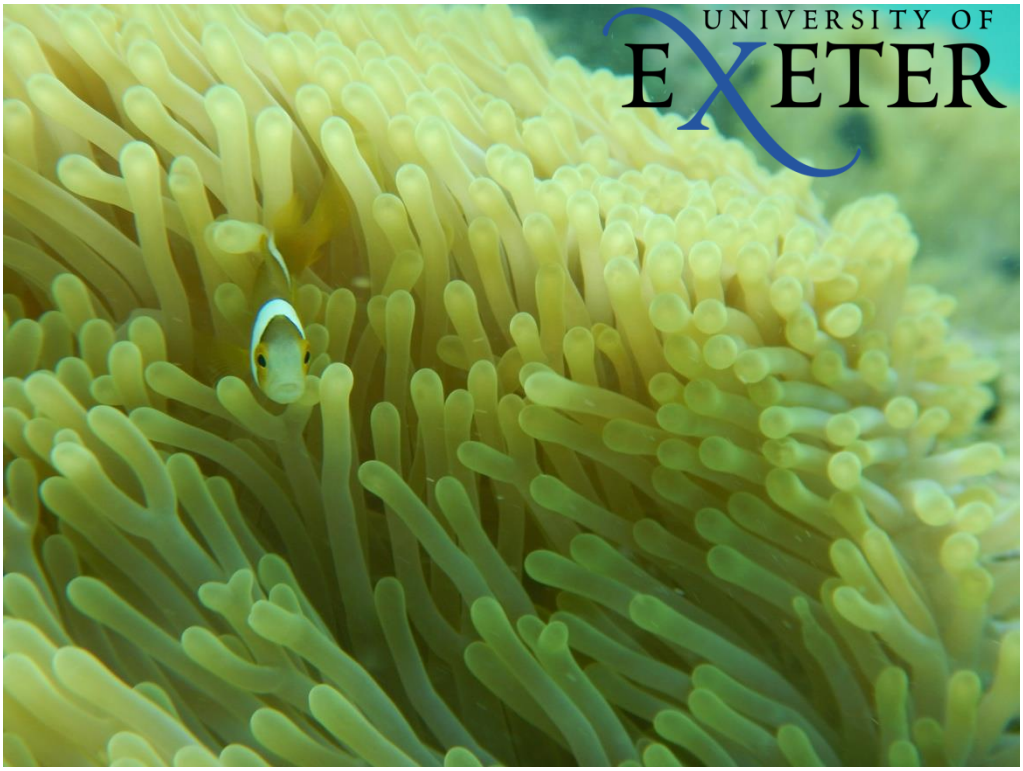
# HEARING NEMO: ALARM CALLING BEHAVIOUR IN A CORAL REEF FISH

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## Abstract

Alarm calling behaviour—the production and use of particular vocalisations that warn of impending danger, and the response to those vocalisations—is a major anti-predator strategy seen in a wide range of bird and mammal species. Receivers of alarm calls that respond adaptively to these acoustic signals obtain fitness benefits through increased survival. Although fish are known to be highly vocal and to use acoustic signals and cues to obtain information about the environment, the potential use of alarm calls has received little empirical attention. This study adapts established terrestrial experimental protocols—combining video and audio recordings and detailed observations with simulated model predator attacks and resulting call playbacks—to explore alarm calling behaviour in the orangefin anemonefish (*Amphiprion chrysopterus*) in Moorea, French Polynesia. The use of ecologically relevant predator models in this study illuminates the evasive and wary/agitated anti-predator behavioural responses of this highly social species, including on the overall rate of vocalisation. Despite a high degree of variation in responses across individuals and life stages, there was also a trend towards increased response to the initial onset of alarm call playback in isolation of any visual stimulus. Thus, *Amphiprion chrysopterus* individuals demonstrate the three recognised stages of alarm calling behaviour: call production, call usage and call response. This research deepens our understanding of fish vocal behaviour, identifying the ecological and social contexts in which different sounds are made, and the responses they elicit. More generally, this research reflects the growing interest in understanding how fish species inhabiting coral reefs interact whilst navigating their acoustic environment.

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# 1. General Introduction

***"What we know is a drop, what we don't know is an ocean"***

***Isaac Newton***

All animals use a range of signals to communicate, navigate their environment, and survive. But there is much we still do not know or fully understand about fish and the marine environment. How fish interpret acoustic signals and integrate that information into decision-making, from the scale of individuals to entire shoals or populations, is important to understand, especially in the context of increasing anthropogenic pressures threatening the stability of ocean ecosystems (Faulkner et al. 2018). Threats such as overfishing, ocean acidification, habitat degradation and noise pollution interact and influence the environment and sensory ecology of many marine species (Putland et al. 2017). This project aims to develop a better understanding of communication in coral reef fish and their use of acoustic signals, which form part of the soundscape within coral reefs (Radford et al. 2014). Alarm calling behaviour, a key anti-predator strategy, has direct effects on the survivorship of individuals and is well established in terrestrial contexts (Goodale et al. 2008; Magrath et al. 2015; Morris-Drake et al. 2017). Alarm calling in fish, however, remains greatly understudied despite the intense predator–prey overlap in coral reef environments. In this introductory chapter, I consider what part alarm calls play in the soundscape of a "choral reef" (Lobel et al. 2010). I begin by exploring alarm calling behaviour in principle and its evolutionary development, followed by examining the physiological capacity of fish to hear and vocalise. I then look at known acoustic communication in fish and the environmental context of the coral reef for such communication. I also consider alarm signalling in fish, and the limited amount of work that has investigated acoustic alarm signals in that taxa.

## 1.1 Alarm signalling

Animals communicate as a means of gathering information about the world they inhabit, facilitating adaptive decision making (Magrath et al. 2015). For communication to have evolved, it must improve fitness for both the signaller (sometime called the sender) and the receiver, where the signaller benefits from

the behavioural response of the receiver (Myrberg, 1981; Magrath et al. 2015). Alarm signalling involves the production of a warning about danger which elicits adaptive anti-predator strategies in receivers. Such signals are used in the context of predator–prey interactions, predation being one of the strongest selective forces affecting the fitness of an individual (Hesse et al. 2015). Having evolved across multiple taxa (including mammals, birds and fish), alarm signalling improves fitness in species across varied biotic and abiotic environmental pressures.

There are at least 16 different hypotheses accounting for the evolution of alarm signals through the benefits to the signaller or its kin, highlighting the challenge establishing which selective pressures are involved in each particular case, especially as benefits may not be directly apparent (Smith, 1992). The potential adaptive advantages of alarm calling include startling the predator, attracting mobbers from kin or reciprocal species, warning kin in an altruistic act or even attracting secondary predators, changing predators to prey (Hogstedt, 1983). The benefits of alarm signals are often obvious for conspecific receivers (whereby they adjust or alter their behaviour to avoid predation), and as such benefits often go beyond a few nearby and closely related group members, alarm signals have the capacity to provide adaptive benefits indirectly through phenomena such as inclusive fitness (kin selection), reciprocal altruism and cooperation (Smith, 1992; Hollén & Radford, 2009; Meuthen et al. 2014). Arguably, the act of communicating through alarm signals implies a shared code between those interacting and thus the cost to the signaller to accomplish their specific aim must be less than the cost needed without the signal (Myrberg, 1981). Inclusive fitness suggests that cooperation of an individual with its kin, its close genetic relatives, could increase kin survival and thus would increase the fitness of an individual indirectly through the propagation of their shared genetic material (Meuthen et al. 2014). This is suggested by Waldman (1982) in that the tendency of the toad tadpole (*Bufo americanus*) to form kin associations can increase their inclusive fitness when alarm signalling in response to predator attacks. Alarm signalling individuals often increase their conspicuousness to predators and/or reduce their foraging time by increasing vigilance behaviours, yet when groups consist of highly related individuals, efficient vigilance systems benefit the individual through inclusive fitness (Rasa,

1989). Non-related individuals, such as in mixed-species bird flocks, have evolved alarm calling behaviour as cooperative strategies that “out-compete” selfish alternative strategies, when individuals interact often through benefits broadly recognised as predator evasion and increased foraging efficiency (Terborgh, 1990; Connor, 1995; Sridhar, 2009). This is especially likely when either the mixed groups are at risk from similar predatory species, benefitting most from adaptive cooperative behaviours and mutualisms, or the environment is particularly uncertain and variable (Uitdehaag, 2011).

Alarm signalling can take many different forms and follow different sensory pathways. The senses used to detect and communicate predation risk depend much on the behavioural ecology of that species (Caro, 2005). Ungulates, for example, display a diverse range of anti-predator behaviour such as the visual tail-flagging in white tailed deer (*Odocoileus virginianus*) (Caro et al. 1995). Chemical alarm signals have been recorded across many taxa. An example by Hews (1988) describes the western toad larvae (*Bufo boreas*) increasing activity and predator avoidance upon detection of chemical alarm substances from an injured conspecific. Acoustic alarm signals have evolved in many bird and mammal species (Caro, 2005). For example, alarm calls have been described when sentinel mongooses vocalise to conspecifics signalling predator presence (Kern & Radford, 2013).

## 1.2 Alarm calling behaviour

Auditory alarm signals, or alarm calls, form part of the communication system to signal danger, acting as a key anti-predator strategy (Smith, 1992; Hollén & Radford, 2009). Vocal communication allows signallers and receivers to be out of sight and does not require the suspension of other activities (Kern & Radford, 2013). Alarm calling involves the recognition of a hazard by the signaller, the generation of a signal that is recognised by the receiver, and the appropriate behavioural performance by the receiver to avoid danger (Smith, 1992). Hollén & Radford (2009) describe the three main aspects of alarm calling behaviour as call production (with specific acoustic features), call usage (in particular contexts) and call response which can be either unconditioned (a direct response to the energy of the signal itself) or conditioned (resulting from past interactions where signals are related to certain responses). A study by Schel et

al. (2013) using a model python predator demonstrated both alarm call production and alarm call use in chimpanzees (*Pan troglodytes*) in which the calling only stopped once recipients were safe from the predator. In addition, playback experiments of alarm calls on vervet monkeys (*Chlorocebus aethiops*) demonstated that exposure to an acoustic signal alone stimulated specific adaptive behavioural responses to avoid particular predators (Seyfarth et al. 1980). Numerous studies have looked at alarm call production, use and response in terrestrial organisms, and there are many examples where model predators have been used to demonstrate alarm signalling behaviour in birds, mammals and lizards as well as playback experiments with most showing anti-predator behaviours (Hollén & Radford, 2009; Magrath et al. 2015).

Magrath et al. (2015) categorised vocal alarm calls depending on the behavioural response they induce: a “flee alarm call” is prompted by a hunting predator often posing immediate danger; a “mobbing call” is triggered by a predator that does not pose an immediate threat; and a “distress call” is often used after an individual is attacked or captured. This illustrates that the exchange in alarm call and resulting anti-predator response between signaller and receiver are adaptive due to their specificity and by being flexible, modifiable and cognitively rich (Seyfarth & Cheney, 2010). Alarm calls can often communicate more than just a simple warning of danger, providing additional information such as the predator type, predator behaviour and the urgency of the threat which influences the behavioural responses of receivers (Hollén & Radford, 2009). For example, the seminal work on vervet monkeys demonstrated their capacity to convey acoustically the type of predator in their alarm calls (Seyfarth et al. 1980; Seyfarth & Cheney, 1986). Receivers were demonstrated to respond appropriately to these specific alarm calls, looking upwards for “eagle” alarm calls and retreating into trees on hearing “leopard” alarm calls (Seyfarth & Cheney, 2010).

The capacity of organisms to learn from experience enables individuals to modify alarm calls to remain relevant and effective across various life stages and environmental contexts. Identifying and classifying predators has been shown to improve with age, demonstrating the conditioning of individuals over time (Seyfarth et al. 1980). For instance, white-browed scrubwren (*Sericornis*

*frontalis*) nestlings learn to adapt their calls for their stage of development (Platzen & Magrath, 2005).

As well as the intended recipients of alarm calls, it is now clear that other species can gather relevant information through “eavesdropping”; an acoustic interceptive behaviour in which species gather important information from signals intended for others. Eavesdropping has been experimentally explored across 70 vertebrate species (Magrath et al. 2015). In the sympatric species of mormyrid fish (*Pollimyrus isidori* and *Brienomyrus niger*) the non-vocal *B. niger* mid-brain neurons responded to click trains of *P. isidori*, suggesting an adaptive advantage to heterospecific eavesdropping of a vocal species by a non-vocal species (Smith, 1992). This can be beneficial by either prompting anti-predator responses or providing a learning opportunity about predators, and thus heterospecific alarm calling can provide a network of information about danger at a reduced cost, even to species that are solitary or differ in family, order or class from the original signaller (Magrath et al. 2009; Magrath et al. 2015). Eavesdropping behaviour is likely to persist in contexts where the heterospecific “interceptor” that *overheard* a signal does not reduce the adaptive advantage to the conspecific receiver *hearing* the signal, thus avoiding the introduction of a selection pressure for the signaller to cease alarm calling by altering any post-signal benefits (Myrberg, 1981; Smith, 1992; Magrath et al. 2009). Both acoustic similarity and learning in sympatry (species co-occurring in the same geographical location) are important in enabling heterospecific recognition of alarm calls and learning is increasingly considered essential to enabling recognition of the diversity of alarm calls (Magrath et al. 2009; 2015).

Not all alarm calls are an honest signal. Indeed kleptoparastitic species—animals that join other groups to steal prey—are known to give false alarm calls (Camphuysenl & Webb, 1999; Hollén & Radford, 2009). In such cases a kleptoparasitic signaller benefits from available food resources following the response of a receiver and this in turn influences the efficacy and reliability of an alarm call (Hollén & Radford, 2009). For instance, fork-tailed drongos (*Dicrurus adsimilis*) make false alarm calls (that are structurally indistinguishable from the true alarm calls) when they see the receiver handling food in order to steal it once the receiver has retreated for cover (Flower, 2011).

In general, alarm calling has been extensively studied in terrestrial systems, with the majority of research focussing on mammals and birds. Fish, however, are increasingly known to benefit from such adaptive anti-predator strategies, forming effective examples of different forms of alarm signalling. The more we learn, the more we realise that fish show many of the same capacities for acoustic activity as birds (Winn et al. 1964) and therefore there is potential for similar alarm calling behaviour.

## 1.3 Capacity and extent of acoustic communication in fish

Fish form the largest group of vertebrates that vocalise and depend on acoustic signals for various social interactions (Amorim et al. 2015). The field of marine acoustic ecology, however, remains comparatively new; Charles Breder (1968) (in Lobel et al. 2010) commented that the study of fish behaviour '*has usually been treated as though fish were both deaf and mute*' (p. 329). Yet there has been increasing progress and momentum in the study of fish vocal communication in the last 50 years (Lobel et al. 2010). Thousands of fish species inhabit coral reefs worldwide and the production of sound has been described in 300 of these species, increasing to 800 species ocean-wide (Slabbekoorn et al. 2010; Tricas & Boyle, 2014). The acoustic capacity of fish, sometimes analogous to that of birds, can be noted in the dawn chorus, where increased acoustic activity is detectable during the early morning within a coral reef (Parsons et al. 2016). Although there are many examples of fish losing vision, as far as is known, all fish retain their auditory sense (Lobel et al. 2010). The benefit and adaptive significance of accessing the audible world seems especially likely in an environment where sound travels five times faster than in air (about 1500 vs 300 $ms^{-1}$) and attenuates more slowly than in air, meaning that fish calls can potentially propagate hundreds of metres to kilometres (Lobel et al. 2010; Slabbekoorn et al. 2010; Holles et al. 2013; Parsons et al. 2016).

Most sound production in fish has been associated with either reproduction or aggression (Lobel et al. 2010). Studies in fish have focussed on acoustic communication in relation to mate choice or territorial defence. In damselfish, one of the best studied fish families for courtship and agnostic interactions, individual chirp variation in male damselfish influences partner selection for

spawning and “keep out” vocalisations acoustically delineate their territories to conspecifics (Myrberg & Riggio, 1985; Myrberg, 1997; Colleye & Parmentier, 2012). Lobel et al. (2010) reviewed studies of the acoustic communication of fishes, summarising that the focus has mostly been on:

a) The physiology and biology of sound production, especially how acoustic signals are affected by functional morphology. For example, Bass & McKibben (2003) illustrated that the frequency sensitivity of the hearing organ (sacculus) in midshipman *(Porichthys notatus)* and toadfish *(Opsanus beta)* correlates with the frequencies of vocalisations that are behaviourally relevant. It is often the case, however, that the bioacoustic capacity remains only partially characterised for fish (Tricas & Boyle, 2014).
b) The specificity and repeatability of call patterns and behaviours, especially in the context of reproduction; such studies have often explored whether such behaviours are successfully initiated with playback techniques. For example, acoustic signals have been recognised as an essential component of the reproduction of midshipman and toadfish (Bass & McKibben, 2003). In addition to reproduction, understanding the acoustic characteristics and temporal patterns of particular vocalisations in choruses and their repeated locations would provide significant information for long-term monitoring of vocal fishes and their ecosystems (Parsons et al. 2016).
c) The potential for heterospecific responses and whether sympatric species respond differently to playback of conspecific sounds. Any differences between individuals or species would highlight interesting influences of sound in assortative mating, mate choice and reproductive isolation of populations. Indeed, Lobel et al. (2010) suggest that aspects of acoustic signals such as pulse repetition rate form a basis for interspecific recognition.

Hearing is thought to have evolved in part as a mechanism to monitor the ambient soundscape, enabling the detection of potential predators or prey (Lobel et al. 2010). Fish species vary in the spectral range to which they are sensitive depending on the presence or absense of particular evolved

morphological structures (Slabbekoorn et al. 2010). The neural network required for vocalisations can be fixed when very young as seen in male midshipman where juveniles commit to specific vocal phenotypes, influencing the growth and development of their acoustic motor system (Bass & McKibben, 2003).

Hearing structures in teleost fish are like other vertebrates, the inner ear has three semi-circular canals and three organs making up the otolithic canal—the sacculus, utriculus and lagena—and these organs respond directly to movement by the fish and particle motion in their medium (Bass & McKibben, 2003). There can also be indirect stimulation through acoustic pressure changes that can be detected by gas-filled chambers such as the swimbladder. On a neurophysiological level, studies of teleost fish have identified brainstem populations that mainly enable the encoding of acoustic information, suggesting teleost fish have central pathways that are dedicated to hearing (Bass & McKibben, 2003). Fish are one of the only vertebrate groups in which individual neurons in the hindbrain have been identified to establish the physical attributes of a vocalisation directly, whereby the vocal pattern generator within the caudal medulla is activated by the vocal–acoustic pathway and sonic muscles are triggered by occipital nerves to form highly stereotyped vocalisations (Bass & McKibben, 2003).

Some fishes, including elephantfishes (mormyrids) and the gadoids cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*) vocalise through a sonic swimbladder mechanism (Hawkins & Amorim, 2000), and Bass & McKibben (2003) suggest the same is likely for damselfish. For such species, sonic muscles are attached to the lateral walls of the swimbladder and neuron activity leads to synchronised firing of the sonic neurons on both sides, resulting in concurrent contraction of the sonic muscles (Bass & McKibben, 2003). The swimbladder often seems to be involved as a resonating device for loud vocalisations and the pharyngeal jaws can also be involved in sound production (Lobel et al. 2010). Studies of yellowtail clownfish (*Amphiprion clarkii*) demonstrate that the sound is produced by the rapid closure of the upper and lower jaw and teeth (Lobel et al. 2010). Closely related skunk clownfish (*Amphiprion akallopisos*) have agonistic vocalisations that form different

“dialects” depending on their geographic distribution (Parmentier et al. 2005). Vocalisations can therefore be influenced by morphological variations of physiological features, geographical location and the acoustic habitat (Bass & McKibben, 2003; Parmentier et al. 2005; Lobel et al. 2010).

As fish clearly possess the physiological capacity to vocalise, it is important to consider which aspects of fish vocalisations exhibit qualitative and quantitative variation (Parsons et al. 2016). Specific calls may have particular vocal characteristics that enable them to be isolated from other vocalisations. Parmentier et al. (2016) studied damselfish, a focal group for studying reef fish vocalisation, by breaking down calls and their associated behaviours. Many vocalisations occur in a series of pulses and therefore a series of parameters can be used to categorise particular vocal patterns (Parmentier et al. 2016). For a single vocalisation, one can measure:

1. number of pulses in a series;
2. pulse duration;
3. interpulse interval (silent period between pulses);
4. pulse period (time between the start of one pulse and the next);
5. pulse repetition rate between pulses within a single series (number of pulses per unit time);
6. frequency or power spectrum (output of the amplitude, often given in dB re 1 μPa, plotted across frequencies); and
7. dominant frequency of the pulse or over the entire pulse series.

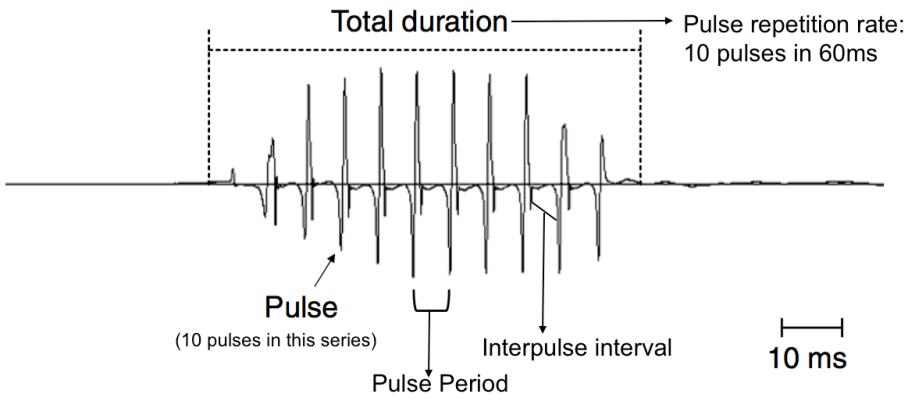



**Figure 1.1** Edited oscillogram from Vasconcelos & Ladich (2008) of temporal sound characteristics in single grunt of Lusitanian toadfish (*Halobatrachus didactylus*).

Acoustic properties can be analysed visually with spectrograms (frequency-intensity vs time), oscillograms (amplitude vs time; e.g. Fig. 1.1), and frequency spectra (amplitude vs frequency) (Parmentier et al. 2016).

Many studies have explored communication in fish across reproductive and antagonistic contexts, identifying the underlying physiological mechanisms as well as the behaviours associated with such acoustic signalling. It is somewhat surprising, however, that few studies have explored how fish, with good hearing and sound producing ability, use such an acoustic sense in the context of predation risk (Myrberg 1981).

## 1.4 Alarm signalling in fish

A predator threat can influence the behavioural decision-making of potential prey in different ways: it can trigger an alternative behaviour such as mobbing or retreating to shelter, and can influence a current behaviour such as level of vigilance or movement when feeding and foraging (Smith, 1992; Larson & McCormick, 2005; Hollén & Radford, 2009). It can also result in alarm signalling, which may benefit shoals of conspecifics, and this is especially the case with kin due to inclusive fitness (Meuthen et al. 2014). This is seen in the cooperative predator inspection by juvenile cichlid fish (*Pelvicachromis taeniatus*), in which certain individuals undertake high-risk predator inspections to assess and communicate danger for the benefit of the shoal (Hesse et al. 2015). This study continues by demonstrating that such cooperative inspection behaviours increase significantly when the individuals are related (Hesse et al. 2015). Benefits of alarm signalling do not, however, remain limited to conspecific receivers. As in mixed species bird flocks, heterospecifics in multi-species aggregations of fishes may have evolved to benefit from the alarm signalling behaviour of other species to increase predator evasion and increase foraging efficiency (Terborgh, 1990; Connor, 1995; Sridhar, 2009; Lobel et al. 2010).

Communication in the context of predation is increasingly well established in fish as alarm signalling, across different sensory modes, increases survivorship for both the signaller and receiver. Visual alarm displays have been reported in gobies or the bioluminescent alarms of batrachoidids (Smith, 1992). Chemical

alarms have been described by Larson & McCormick (2005) in the response to predator odours in damselfish. Sound forms an important mode of signalling in fish, especially as sound may be involved within the small inter-individual distances through vibratory signals and particle displacement in fish schoals, enabling rapid communication within a group (Myrberg, 1981). It should not therefore be surprising that, in highly social groups such as schools or aggregations, sound production can form an essential alarm signal. Studies show that, during cooperative anti-predator manoeuvres, individuals responded to the fright responses of others despite being unable to sense the predator themselves (Myrberg, 1981; Smith, 1992).

It is important to note that the categorisation of vocalisations varies across academic research. Many studies include the behavioural response of sound production when either fleeing, confronting or attacking a potential predator within the same category of “agonistic behaviours” as feeding competition, territory defence, combat and intra/interspecific chases (Lobel et al. 2010). This highlights that whilst alarm calling and the resulting behavioural response have not been explicitly studied extensively in fish, elements of this behaviour may previously have been described in many acoustic studies.

## 1.5 Current knowledge of alarm calling in fish

Alarm calling has become more established for terrestrial ecological systems such as birds and mammals over the last decade (Hollén & Radford, 2009), but it remains understudied within aquatic systems. Thus far, fourteen families of fish are known to produce sounds in response to being disturbed, three of them specifically in response to predator presence (Myrberg, 1981; Lobel et al. 2010). Most sounds made due to disturbance are produced by structures independent of the defence mechanism itself and are therefore not simply an acoustic by-product (Lobel et al. 2010). It therefore seems likely that many fish species would adaptively benefit from alarm calling in response to predation, especially in species that are particularly vulnerable to predators that possess advanced hearing specialisations and sound producing capabilities (Smith, 1992; Lobel et al. 2010).

While predominantly qualitative, some evidence of alarm calls can be found in fish with appropriate behavioural responses (Table 1.1):

**Table 1.1:** Published studies that explore elements of alarm calling behaviour in fish.

| **Species making alarm call** | **Behavioural context and response** | **Reference of study** |
|---|---|---|
| Cod (*Gadus morhua,* Gadidae) | Made grunting vocalisations when startled, chased or cornered. | Brawn, 1961 |
| Longspine squirrelfish *(Holocentrus rufus,* Holocentridae) | Produced a specific alarm ”staccato” when encountering a large fish moving on their level (spotted moray *Gymnothorax moringa*).<br>The call induced conspecifics either to withdraw into a refuge or to mob the potential predator. | Winn §et al., 1964 |
| Menpachi squirrelfish (*Myripristis berndti,* Holocentridae) | Being confronted by divers, eels or playbacks of recoded “staccato” calls elicited either investigation approaches or rapid withdrawal into a refuge. | Salmon, 1967 (in Smith, 1992) |
| Nassau grouper *(Epinephalus striatus, S*erranidae*)* | The use of sudden loud sounds to potentially startle predators enabling escape or preventing capture (analogous to the use of visual flash displays in other species). | Myrberg, 1981 |

Winn et al. (1964) provides one of the only examples of a study that explicitly explores alarm-like sounds and the behavioural responses produced when exposed to various playbacks. Longspine squirrelfish modified their behaviour in response to sounds played underwater. The fish were found to “jump”, retreat into crevices, or investigate its source, and such behaviours were not triggered by lobster sounds used as a control (Winn et al. 1964). Discrimination between different sounds playbacks was considered poor, however, and it is difficult to conclude what aspects of the sounds produced in a lab setting were triggering the recorded responses in these studies. These studies provide valuable insight, but further research is required to test conclusively for alarm calling behaviour.

Alarm calls could communicate information with a potentially high degree of specificity and flexibility, especially as fish show evidence of acoustically mediated recognition of individuals (Myrberg & Riggio, 1985). Winn et al. (1964) described differentiation in the “staccato sound” of the longspine squirrelfish dependent on the type of stimulus encountered: a spotted moray (*Gymnothorax moringa)* or a palometa (*Trachinotus goodei)* induced conspecifics to approach the signaller or withdraw into a refuge. Such staccato sounds have also, however, been noted to be produced in other behavioural contexts (such as to conspecifics in Winn et al 1964) making it challenging to know cause and effect. The starry goby (*Asterropteryx semipunctatus*) found in coral reefs can learn to associate danger with novel chemical alarm cues that then trigger a typical alarm response, demonstrating the capacity of fish to learn with experience (Larson & Mccormick, 2005). In addition, naïve salmonids show lower vulnerability when mixed with experienced fish, indicating the learning and transfer of predator avoidance behaviours (Smith, 1992). These examples demonstrate the capacity for learnt behaviours and the association of signals related to predation in fish. It is therefore likely that behavioural responses to alarm calling are influenced by the environmental variables affecting predation levels on the signalling individual.

In summary, virtually nothing is known on acoustic alarm signalling in fish despite recognition of its potential importance and value since the 1960s (Salmon, 1967; Myrberg, 1981). Auditory alarm signals as well as tactile and lateral line signals have also been noted in several fish species and this broad range of alarm and distress signalling has been noted in upwards of 24 different fish families (Myrberg, 1981; Smith, 1992). Conclusive causal links between acoustic alarm signalling and resulting behavioural responses in fish, however, remain untested.

## 1.6 In the context of coral reefs

The soundscape of an environment is influenced by many sources of acoustic stimuli. These can be biological (‘biophony’), physical (‘geophony’) or due to anthropogenic sources (‘anthrophony’) (Nedelec et al. 2015). Studying any vocalisations requires a degree of understanding of the surrounding acoustical

context, including how that influences vocalisations themselves and thus marine animal behaviour, ecology and evolution (Lobel et al. 2010). In environments such as coral reefs, which cover no more than 0.1% of the ocean floor but host 25% of all known fish species, biophony often dominates the soundscape (Moberg & Folke, 1999; Nedelec et al. 2015). This comes as no surprise when, at particular diurnal periods, the biophony can cause a shift in ambient noise levels of an entire area, with fish and invertebrates contributing a significant vocal component to local soundscapes (Nedelec et al. 2015; Parsons et al. 2016). Certain types of fish chorus can cause a shift in the ambient noise (sometimes 41 dB above expected levels in the range 250–3000 Hz). How sounds propagate can help elucidate the characteristcs of certain vocalisations as well indicate the biodiversity of vocal species in the area (Lobel et al. 2010; Nedelec et al. 2015; Parsons et al. 2016). How species communicate acoustically, orientate themselves and locate suitable habitat depends upon the context of their soundscape and the biophony present in their environment (Freeman & Freeman, 2016; Simpson et al. 2016). The sounds that make up the baseline acoustic environment are both biologically and ecologically important, directly influencing survivorship at population levels (Simpson et al. 2005; Lobel et al. 2010).

Ambient sound within a “choral reef” is biologically and ecologically meaningful, but it can also create interference, limiting the ability of fish to discriminate important sounds, especially as fish sounds are often highly audible and widely propagating (Lobel et al. 2010). To counteract this “masking” (the increased detection threshold of an acoustic signal due to others), fish may adopt temporal and frequency partitioning, as suggested in the ‘acoustic niche hypothesis’, to increase the likelihood of the intended receivers hearing the signal and making the appropriate behavioural response (Parsons et al. 2016). This was seen in work by Nedelec et al. (2015), which found low levels of overlap in acoustic activity between most fish and snapping shrimp, and also in Parsons et al. (2016) when recordings without fish choruses coincided with periods of significant noise from humpback whales (*Megaptera novaeangliae*) and wind. There is, however, a trade-off for vocalising fish between the need to communicate and the risk of being overheard by a predator or competitor (Lobel et al. 2010).

Of the 48 vocal families within the 179 families of fish that inhabit coral reefs, the spectral range in vocalisations on tropical reefs is believed to extend from below 100 Hz to 8000 Hz, and the duration of a basic sound unit can range from 10 ms to 6 s (Lobel et al. 2010). Reef fish must adapt, through interaction between sensory and motor mechanisms, to coastal shallow waters with changing physical attributes (for example soft or hard substrate) combined with a dense overlap of acoustic signals, to enable efficient signalling (Bass & McKibben, 2003). This demonstrates that environments that change physically (such as with season) and biologically are behaviourally relevant to vocalising fish and indeed can be seen in differing dialects between allopatric members of the same species (Bass & McKibben, 2003; Parmentier et al. 2016). Parmentier et al. (2016) found that *Dascyllus* species, a group of sympatric-living reef fish, restrict the variability of their sounds. Indeed, *D. trimaculatus, D. aruanus* and *D. flavicaudus* in Moorea (French Polynesia) had significant differences in the pulse period and interpulse interval from *D. trimaculatus* and *D. aruanus* from Toliara (Madagascar). The acoustic regime shifts across variable coral reef habitats, altering ecological communities and the soundscape as a result (Nedelec et al. 2015; Norström et al. 2016). This demonstrates the intimate coupling of vocalisations and acoustic context in “busy” coral reefs (Lobel et al. 2010; Parmentier et al. 2016).

Coral reefs provide valuable study systems for a vast range of research, including that of acoustic communication and alarm signalling. This in part is due to the richness of soniferous species present in the coral reef matrix, many of which remain site attached and act as tractable study species for field research. Coral reef conditions, including good visibility, often lend themselves to field studies of high ecological relevance. With corals as well as fishes playing multiple functional roles, many different processes can be supported with various study systems (Bellwood et al. 2004). Predation levels strongly influence communities on coral reefs and, as predator–prey interactions have a direct effect on survivorship, influence fish behaviour and alarm signalling between species (Boaden & Kingsford, 2015). This makes coral reefs a valuable space to study alarm calling behaviour.

Clownfish are coral reef fishes that remain in a fixed location due to their mutualistic symbiotic relationship with anemones (Colleye & Parmentier, 2012). In this mutualistic relationship, clownfish are protected from predators by the stinging nematocysts in the anemone tentacles, from which they are protected by a mucus coating (Brooks & Mariscal, 1984; Schmiege et al. 2017). The clownfish, in turn, defend the anemone from predators such as turtles. As a social species, clownfish possess a strict size dominance hierarchy, in which females are the largest and dominant individual, followed by a reproductive male and juveniles of diminishing size. Clownfish are protandrous hermaphrodites, in which the largest male changes sex to female once the dominant and largest individual (the female) migrates or perishes (Parmentier et al. 2009). Thus individual size conveys position in the social hierarchy.

Clownfish are tractable model species as they remain in fixed locations, are highly social and are prolific vocalisers that produce a wide range of sounds (Parmentier et al. 2007). Their vocalisations are also likely to be an honest signal as they correlate with body size as seen in studies showing that smaller males produce higher dominant frequencies and shorter duration pulses than larger counterparts (Lobel et al. 2010; Colleye & Parmentier, 2012). Clownfish species are known to produce sounds in multiple ecological contexts, but studies thus far have only focussed on agonistic interactions within the size-based dominance hierarchy (Parmentier et al. 2007; Colleye et al. 2009; Colleye & Parmentier, 2012).

## 1.7 Thesis aims

Theory on alarm calling and its resulting behavioural responses is much more strongly established in terrestrial systems, from vervet monkeys to scrubwrens (Seyfarth et al. 1980; Magrath, 2015), than in aquatic systems. This project aims to combine the terrestrial knowledge base of this anti-predator strategy with the evidenced capacity of fish to vocalise and produce alarm signals (Myrberg, 1981; Smith, 1992). It aims to apply this to the context of a coral reef environment. This project will therefore explore the behavioural ecology of alarm calling behaviour in coral reef fish by studying the alarm call production, usage and resulting behavioural response of the orangefin anemonefish (*Amphiprion chrysopterus*) in Moorea, French Polynesia. This project, using

underwater audio and video recordings in combination with predator-model and playback experiments in natural conditions, aims to uncover the capacity of this acoustic anti-predator behavioural strategy in *A. chrysopterus* within its ecologically relevant context.

# 2. Alarm Calling Behaviour in a Coral Reef Fish

## 2.1 Introduction

Alarm signalling, a form of communication in which the signaller conveys information about a predatory threat to elicit adaptive anti-predator strategies in receivers, has evolved in multiple taxa including mammals, birds and fish (Myrberg, 1981; Caro, 2005; Hollén & Radford, 2009; Magrath et al. 2015). There are currently around 16 different hypotheses for the adaptive benefits and evolution of alarm signalling, predation being one of the strongest selective forces affecting individual fitness (Smith, 1992; Hesse et al. 2015). Alarm signals can have a direct benefit to receivers whereby they adjust or alter their behaviour to avoid predation, and can also provide adaptive benefits indirectly to signallers through phenomena such as inclusive fitness (kin selection), reciprocal altruism and cooperation (Smith, 1992; Hollén & Radford, 2009; Meuthen et al. 2014). Arguably, the act of communicating through alarm signals implies a shared code between those interacting and thus the cost to the signaller to accomplish their specific aim must be less than the cost needed without the signal (Myrberg, 1981). Alarm signals have been described across visual, chemical and acoustic modes of communication. For example, white-tailed deer (*Odocoileus virginianus*) exhibit tail-flagging behaviour when detecting a predatory threat, whilst larvae of the western toad (*Bufo boreas)* increase activity and predator avoidance behaviours upon the detection of chemical alarm signals (Hews, 1988). However, it is acoustic alarm signals that have received perhaps the most research attention.

Auditory alarm signals, or alarm calls, form part of that communication system to signal danger, acting as a key anti-predator strategy (Smith, 1992; Hollén & Radford, 2009). Alarm calling involves the recognition of a hazard by the signaller, the generation of a signal that is recognised by the receiver, and the appropriate behavioural performance by the receiver to avoid danger (Smith,

1992). There are therefore three main aspects to alarm calling behaviour: call production (with specific acoustic features), call usage (in particular contexts) and call response (Hollén & Radford, 2009). For example, model python predator exposures have been shown to elicit alarm call production and useage in chimpanzees (*Pan troglodytes*), and playback experiments of alarm calls in vervet monkeys (*Chlorocebus aethiops*) demonstated specific predator avoidance behaviours as an alarm call response (Seyfarth et al. 1980; Schel et al. 2013). Alarm calls often convey complex and specific information about the type of predator, its behaviour and the urgency of the threat which influences the behavioural response it elicits in the receiver (Hollén & Radford, 2009). An example of this can be seen in how white-browed scrubwren nestlings (*Sericornis frontalis*) react more strongly to alarm calls that depict a greater danger (Platzen & Magrath, 2005). Virtually all knowledge of acoustic alarm signalling behaviour comes from terrestrial species, and yet acoustic communication is common in fish.

The production of sound in fish has been described in 300 coral reef species, increasing to 800 species ocean-wide (Slabbekoorn et al. 2010; Tricas & Boyle 2014). Although there are many examples of fish having lost their sight, all fish known so far have maintained their auditory sense highlighting the adaptive significance in sensing sound (Lobel et al. 2010). Vocal communication allows signallers and receivers to be out of sight and does not require the suspension of other activities (Kern & Radford, 2013). Moreover, sounds produced by an individual can not only convey a particular signal to the recipient but also provide information on location, size and health of the signaller (Parsons et al. 2016). So far, sounds are known to be produced by either the contraction of sonic muscles attached to the walls of the swimbladder which acts as a resonating chamber or the rapid closure of the pharyngeal jaw and teeth (Bass & McKibben, 2003; Lobel et al. 2010). Specific calls may have particular vocal characteristics such as pulse period and dominant frequency that enable them to be isolated from other vocalisations (Parmentier et al. 2016). Most sound production in fish has been associated with either reproduction or aggression such as the courtship vocalisations in toadfish and the “keep out” territorial vocalisation of damselfish (Bass & McKibben, 2003; Lobel et al. 2010; Colleye & Parmentier, 2012). Acoustic alarm signalling remains greatly understudied in

fish despite recognition of its potential importance and value since the 1960s (Salmon, 1967; Myrberg Jr, 1981).

Thus far, fourteen families of fish are known to produce sounds in response to being disturbed, three of them specifically in response to predator presence (Myrberg, 1981; Lobel et al. 2010). Though these species of fish are known to specifically patterned sounds, both the characteristics of such sounds and the specific behavioural contexts that trigger them remain largely unstudied (Lobel et al. 2010) Most sounds noted to be made due to disturbance are produced by structures independent of the defence mechanism itself and are therefore not simply an acoustic by-product (Lobel et al. 2010). One of the only known examples in response to predators is of the longspine squirrelfish (*Holocentrus rufus*) that produced a “staccato” sound when encountering a spotted moray (*Gymnothorax moringa*) moving towards them and the call induced individuals to withdraw into a refuge (Winn et al. 1964). This study, however, was run in the laboratory, placing fish in troughs and either recording their behaviour from interspecific intrusions or sound playbacks. Certain listed potential “alarm signals” were only recorded when fish in question were hand-held. Building from these initial studies into field experiments would provide valuable insight with additional ecological validity. Many studies include the behavioural response of sound production when either fleeing, confronting or attacking a potential predator within the same category of “agonistic behaviours” as feeding competition, territory defence, combat and intra/interspecific chases (Lobel et al. 2010), and conclusive causal links between acoustic alarm signalling and resulting behavioural responses in fish remain untested.

In this study, alarm calling behaviour was explored in the orangefin clownfish (*Amphiprion chrysopterus*), a soniferous social species of coral reef fish. Coral reefs are home to many soniferous species and the usually clear waters lend themselves to field studies in ecologically valid conditions. Predation levels have a huge influence on the communities of coral reefs and, as predator–prey interactions have a direct effect on survivorship, influence fish behaviour and alarm signalling between fish species (Boaden & Kingsford, 2015). Clownfish are highly vocal species, with previous studies exploring their acoustic communication in the context of reproduction and agonistic interactions

(Parmentier et al. 2007; Colleye et al. 2009; Colleye & Parmentier, 2012). Moreover, clownfish remain in a fixed location due to their mutualistic symbiotic relationship with anemones: the clownfish are protected from predators by the stinging nematocysts in the anemone tentacles (to which they are protected by a mucus coating) and defend the anemone from its predators (Brooks & Mariscal, 1984; Colleye & Parmentier, 2012; Schmiege et al. 2017). The fixed location allows repeated visiting of the same groups and a strict size dominance hierarchy—in which females are the largest and dominant individual, followed by a reproductive male and sub-adult males of diminishing size (Parmentier et al. 2009)—enables the identification of the same individuals in a group. Clownfish are therefore an excellent study species for experimental investigations of alarm call behaviour in natural conditions.

Specifically, the alarm calling behaviour (call production, usage and responses) of *A. chrysopterus* was investigated experimentally in the coral reefs of Moorea, French Polynesia. First, the predatory context was assessed from observational videos. Second, predator models were used to examine anti-predator responses including the production of acoustic alarm signals. Third, behavioural responses to alarm call playbacks were assessed to establish the response to acoustic cues in isolation of visual or chemical stimuli.

## 2.2 Methods

All experiments were conducted at the Centre de Recherche Insulaire et Observatoire de l'Environnement (CRIOBE) research station in Moorea, French Polynesia. Stage 1 involved recording and establishing baseline information on predator occurrence and abundance. Stage 2 was a model-predator experiment to test the behavioural and acoustic responses of *A. chrysopterus* to a predatory attack. Stage 3 was a playback experiment to test the behavioural responses of *A. chrysopterus* to the acoustic stimulus of isolated alarm calls recorded in Stage 2.

### 2.2.1 Stage 1: Ecological predatory context

Initial observations were made at six anemone colonies in which *A. chrysopterus* was present. Video (GoPro4) and audio (Sony PCM-M10, 48 kHz sampling rate, recorder and HTI-96-MIN hydrophone) recordings were made 2–

4 m from the anemone colony. Recordings of 30–45 min were made between 05:00 and 07:00, a window of peak vocal and predator activity (Green, et al., 2011; Parsons, et al., 2016), at each colony during similar wind (light-gentle breeze), weather (sun, no rain) and sea-state (calm-smooth; 0–0.5m wave height) conditions. Videos were subsequently watched using Quick Time Player (Version 10.4 (855) Apple Inc.).

Predator species that entered the video field of view were identified and counted, and the time spent in view was recorded for each potential predator. As predators could re-enter the focal area, repeat counts of the same individual are likely to have occurred. Time spent near the anemone (within video field of view) was therefore noted as an additional metric of predatory threat. Species were assumed to be potential predators if they were known to predate on clownfish or prey of an equivalent size (max. 17 cm). Egg predators were also noted as potential triggers of an acoustic or behavioural response in the adult clownfish. Likelihood of ecological relevance to *A. chrysopterus* as a predator was based on the dietary information available on FishBase (https://www.fishbase.de), “Le Guide des poissons de Tahiti et ses îles” (Bacchet, et al., 2016) and local anecdotal knowledge.

Predators identified within the recordings were categorised under two broad hunting strategies. The “stealth” strategy was defined as predatory species that rely on camouflage, spend prolonged periods of time immobile at anemone sites and often remain within coral reef patches (such as the peacock grouper, *Cephalopholis argus*). The “ambush” strategy included predatory species that are far ranging within both open water and coral reefs, hunt primarily using bursts of speed and tend to be more transient (such as the bluefin jack, *Caranx melampygus*).

### 2.2.2 Stage 2: Vocal and behavioural responses to a predator model

A repeated-measures experiment was carried out, in which each anemone colony (n = 22) received one of three treatments on different days. Experimental anemones were separated from one another by a minimum of 20 m and treatments to the same colony were on separate days to minimise carry over effects. The treatments were exposure to: 1) an ecologically relevant predator

model, 2) a similar sized but non-predatory novel wooden object, and 3) a snorkeler without an object; the latter two treatments acted as controls. Treatments were carried out across each colony in a mixed order established through a Latin Square design with randomisation within sets of six (Table 2.1). Treatment time was kept consistent for a given colony to minimise effects of diurnal variation in behaviour. Two or three GoPro cameras were placed ca. 1.5 m from the anemone at each colony, one facing the anemone and the other(s) facing both the anemone and oncoming snorkeler and model. A HTI-96-MIN hydrophone was also placed ca. 20 cm from the anemone. To synchronise the audio and video from the GoPros and hydrophone, a sound and visual action were made by the snorkeler prior to the acclimatisation period.

**Table 2.1:** Example of the mixed order Latin Squares design for the different treatments over three separate days. Letters indicate which of the three treatments (A = predator model, B = novel object model, C = snorkeler control). Numbers indicate which of the six sleeves the predator model "wore" for that treatment (1 = 1$^{st}$ predator sleeve, 2 = 2$^{nd}$ predator sleeve, etc.). "i" numbering indicates which of the six novel objects was used for that anemone colony (I = 1$^{st}$ novel object model, ii = 2$^{nd}$ novel object model. etc.)

| **Anemone Site** | **Day 1** | **Day 2** | **Day 3** |
|---|---|---|---|
| **Colony 1** | $A^{2}$ | $B^{ii}$ | C |
| **Colony 2** | $B^{iii}$ | C | $A^{6}$ |
| **Colony 3** | C | $A^{5}$ | $B^{i}$ |

All trials included an initial "rest period" of 10 min (7 min acclimatisation and 3 min undisturbed measured behaviour), enabling the clownfish to acclimatise to the recording equipment (as established in Nanninga et al. 2017) without any natural disturbance (e.g. a natural predator attacking the anemone). If there was any natural disturbance, the acclimatisation of 7 min was restarted. There was then a treatment phase (mean duration: 236 s; range 109–461 s) during which the snorkeler was present with or without a model predator or wooden object. Five minutes after completion of the trial, the following additional information was gathered and the equipment collected:

1. any predators spotted within a 2 m radius of the focal anemone;
2. number of *A. chrysopterus* adults and juveniles present at focal anemone;
3. number of *D. trimaculatus* adults and juveniles present at focal anemone;
4. total number of anemones in the territory and how many were bleached; and
5. whether *A. chrysopterus* had a nest and eggs present under the anemone fronds

Domino damselfish (*D. trimaculatus)* individuals were noted due to their frequent presence around the focal anemones. Although they are not as dependent on anemone territories as *A. chrysopterus*, they are often found near such sites and are known to vocalise.

### 2.2.2i Treatment types

The honeycomb grouper (*Epinephelus merra*) was selected as the species to be replicated by the predator model. This selection was based on results from the observational videos in Stage 1: the occurrence of different species, the time they spent near the anemone and the hunting strategy that could be most reliably replicated to elicit an alarm call formed the basis of the selection.

To minimise pseudoreplication, six different individuals of *E. merra* found in the reef surrounding Moorea were photographed. The images were printed to life size (31 cm in length, 8 cm width) on waterproof paper and stuck to the model (a hollow grouper-species silhouette made from fibre glass) with transparent waterproof tape. These images acted as “predator sleeves” covering the predator model (Fig. 2.1).

**Figure 2.1:** Six different *E. merra* sleeves used for the predator model. Pectoral fins were cut open to enable the appearance of free fin movement in the model underwater.

Two ways to elicit a simulated predatory “attack” using the model fish were tested: use of a “pulley” or “puppet” system. The pulley system pulled the predator model towards the anemone with fishing line through an eyehook tethered by weighted stands. This enabled the snorkeler to remain 10 m away and thus avoid snorkeler presence at the anemone. The puppet system attached the predator model to a metal rod with an adjustable length of clear fishing line. The model was attached at both front (base of dorsal fin) and back (tail fin) and was made negatively buoyant by internal 3 x 3 cm sand weights (that avoided rattling noises throughout the trial). This enabled the snorkeler to control the movement of the predator models whilst remaining at the surface. Initial trials found that the predator model did not move naturally or in a reliable way that could be controlled in the pulley system. The puppet system was therefore selected as the preferable method on the basis that the predator behaviour could be more reliably replicated. The length of fishing line was adjusted so that the model would remain on the same level as the anemone, approaching the *A. chrysopterus* colony in a series of standardised movements (Table 2.2) to keep the trials consistent between sites and between treatment types.

The novel object exposure used the same set up as the predator model. The novel object consisted of one of six pieces of wood, 8 cm in diameter and cut to the same length as the predator model (31 cm), and weighted with a 1 kg dive weight. The snorkeler swam with one of the six novel model pieces of wood and conducted the standardised movements at the anemone colony (Table 2.2). For the snorkeler control, the snorkeler swam to the anemone colony conducting the same standardised movements with the metal rod without a model attached to it.

**Table 2.2:** The series of standardized movements with which the snorkeler moved either the predator model or wooden object towards and at the anemone colony during the trial.

| Order | Action |
|---|---|
| 1. | Approach anemone within camera view, keeping model level with anemone. |
| 2. | Circle anemone with model above anemone and wait 10–15 s. |
| 3. | Lower model and quickly strike up towards *A. chrysopterus* with arm jolt. Repeat 3–5 times. |
| 4. | Retreat 2–3 m from anemone, remain in sight of *A. chrysopterus*, wait 1 min. |
| 5. | Swim forward with model as fast as possible and jolt model up towards anemone 3–5 times, following any *A. chrysopterus* that move. |
| 6. | Retreat with model and swim a minimum of 10 m away for post-exposure filming. |

### 2.2.2ii Behavioural response of clownfish

*A. chrysopterus* individuals within each anemone colony were categorised into female, male and sub-adult based on the dominance size hierarchy of the species; female and reproductive male are largest and second largest of the colony respectively, smaller individuals are the non-reproductive sub-adults or juveniles (Colleye et al. 2011). For each colony across all three treatments, each individual *A. chrysopterus* present at the focal anemone was identified by size.

Video footage was analysed in Quick Time Player (Version 10.4 (855) Apple Inc.). The responses to the treatment were categorised into nine different behavioural measures and their duration noted in certain cases (Table 2.3). For each trial, the presence of other potentially soniferous species was noted (measured as species present in cameras field of view).

**Table 2.3:** Definition of different behavioural measures extracted from video recordings.

| **Behavioural measure** | **Definition** | **Output** |
|---|---|---|
| 1. Into/in anemone | Time spent in bodily contact with anemone | Time (s) |
| 2. Towards anemone | Swimming closer to anemone from current position | Yes/No |
| 3. Abandon anemone | Leaving contact with anemone and swimming away from model | Yes/No |
| 4. Away from model | Remaining either within or outside anemone and moving away from model | Yes/No |
| 5. Agitated within anemone | Time spent engaged in high speed swimming in ≥3 directions in quick succession within anemone | Time (s) |
| 6. Head nodding | Time spent facing model and moving head and upper body up and down ≥3 times | Time (s) |
| 7. Agitated outside anemone | Time spent engaged in high speed swimming in ≥3 directions in quick succession outside anemone | Time (s) |
| 8. Approaches or attacks snorkeler/model | Swimming towards model once or repeatedly. | Yes/No |
| 9. Chases *D. trimaculatus* | Chasing one or more *D. trimaculatus* during the trial | Yes/No |

Audio recordings were analysed in Audacity (2.1.2 1991 Free Software Foundation). Sound clips were put through a low pass filter of 1000 Hz at 48 dB and amplified by 5.74±0.64 dB (mean±SE). Any vocalisations were tallied and time-stamped per colony, not separating sounds by source (females, males and sub-adult or species). Vocalisations were further categorised into whether they occurred when the predator model or novel object was present versus absent from the anemone colony during the standardised movements in the trial (see Table 2.2). To account for variation between individuals, the difference in the rate of vocalisations when the model was present/absent was calculated between treatments per colony.

### 2.2.2iii Data analysis

The proportion of time spent in the anemone combined all the trial time periods in which the clownfish was in contact with the anemone. The rate of abandoning the anemone and moving away from the model were categorised as evasive behavioural responses. The proportion of trial time that fish spent agitated combined the measurements of agitated behaviour taken within and outside the anemone during the trial. Both the proportion of time spent agitated and proportion of time spent head nodding were categorised as wary/agitated behavioural responses. These metrics were compared for each treatment in a repeated-measures design for all individuals as well as females, males and sub-adults. Differences in responses between the three treatments were tested with repeated-measures ANOVAs; where significant effects were found, paired t-tests were used to determine which treatments differed from one another. Parametric tests were used following checking of the assumptions of normality and homogeneity of variances.

## 2.2.3 Stage 3: Behavioural response to acoustic playback

The same anemone colonies as for Stage 2 (n = 22) were exposed to two 10 min playback treatments: 1) putative alarm calling vocalisations and 2) ambient reef sounds. Each anemone colony received the two treatments on consecutive days in a counterbalanced order. Both treatments were made up of recordings from Stage 2 of the experiment. At each colony, two GoPro cameras (GoPro4) were placed ca. 1.5 m from the anemone, both facing the anemone at different angles. A loudspeaker (UW-30, frequency response 0.1–10 kHz, University Sound, Columbus OH) was placed 1 m from the anemone. Five playback tracks were made per treatment and randomly allocated to each anemone colony. As vocalisations were not available from all 22 colonies, a colony was not exposed to playback of recordings made at that particular colony.

Once the playback track was started, snorkelers moved a minimum of 20 m away from the anemone colony. An initial “rest period” of 7 min was incorporated into tracks of both treatments by playing recorded ambient non-disturbance reef sounds, thus enabling clownfish to acclimatise to the equipment (Fig. 2.2). This was followed by a 15–25 s acoustic clip of isolated potential alarm calls for the alarm treatment (amplified by 6.66±1.00 dB,

mean±SE) or ambient reef sound (amplified by 12.14±2.61 dB) for the control treatment. Each recording across both treatments ended in a further 3 min of ambient sound to enable the recording of post-exposure behaviour. Acoustic recordings were edited and the playback tracks made in Audacity 2.1.2.

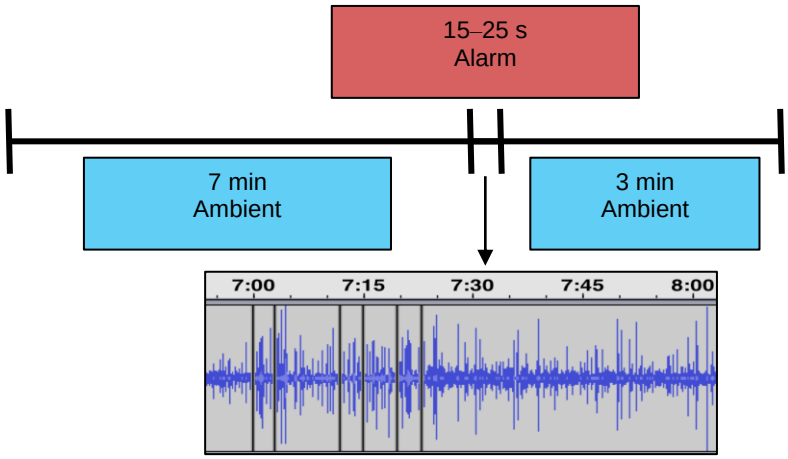



**Figure 2.2:** The content of an alarm playback track in which a potential alarm call recorded in Stage 2 is inserted into the sound file after 7 min and before a further 3min of ambient playback.

### 2.2.3i Measuring behavioural responses

Video footage of both playback treatments were analysed without volume so that the assessor was "deaf" to the sounds present in the playback track (i.e. blind to the treatment). Behavioural responses were collected for females, males and sub-adults (if present) for two time periods: 1) over a 2 min period to incorporate possible behavioural responses both during and after the alarm call and corresponding ambient sounds; and 2) over 25 s (the maximum duration of an alarm call clip) to measure response during the alarm call and avoid possible dilution of the behavioural responses over time (Table 2.4). Behavioural measures across both 2 min and 25 s were also measured prior to the onset of the alarm call clip or corresponding ambient clip, enabling before/after comparisons to be made for each individual. Further analysis, however, concentrated on the 25 s trials rather than the extended 2 min datasets, focusing on immediate behavioural responses as opposed to incorporating post-exposure behavioural responses as with the 2 min analysis. This was done because behavioural responses were very short in duration with very fine-scale differences between treatments and incorporating post-alarm call analysis would dilute any behavioural responses measured.

**Table 2.4:** Different behavioural measures and their definitions.

| Behavioural measure | Definition | Output |
|---|---|---|
| 1. Initial response | Initial response to first 5 s of alarm/ambient clip categorised as 1) towards anemone, 2) into anemone, and 3) no response | Categorical |
| 2. Proportion of time outside anemone | Time and duration taken to retreat to safety (into anemone or associated shelter) per individual throughout 2 min and/or 25 s | Proportion |
| 3. Rate of head turning | The number of head turn movements ≥90° from starting position | Rate |

### 2.2.3ii Data analysis

Treatment differences in the categorical behavioural response to the first 5 s of playback were tested with a McNemar test in which 'towards anemone' and 'into anemone' counts were combined and then compared to 'no response'. Both the proportion of time during the trial spent outside the anemone and rate of head turning were compared between treatments using paired t-tests for all individuals as well as females, males and sub-adults separately. Parametric tests were used after checking the assumptions of normality and heterogeneity of variances.

## 2.3 Results

### 2.3.1 Stage 1: Ecological predatory context

Observational videos across six anemone sites showed 144 occurrences of 19 different species that could potentially act as predators of *A. chrysopterus* or their eggs (Fig. 2.3). Just under half (47%) of all predators were from the families Serranidae (groupers), Labridae (wrasses) and Balistidae (triggerfish) (Fig. 2.3a). In terms of total time spent around the anemone, groupers were most common (32% of observed time), with triggerfish being the next highest (18%) (Fig. 2.3b). This demonstrates that grouper species were most abundant (overall occurrence) and spent the most time in proximity to the anemone being observed (time per individual). The honeycomb grouper (*Epinephelus merra*)

was the most abundant and commonly present of these species and thus was selected as the species to be replicated as the predator model in Stage 2. No natural predation events on *A. chrysopterus* (at any life stage) were recorded throughout the observational recordings.

### 2.3.2 Stage 2: Vocal and behavioural responses to the predator model

The proportion of time spent within the anemone by *A. chryspoterus* did not differ significantly in response to exposure to the predator model, novel object model or snorkeler presence alone (repeated-measures ANOVA, female: $F_{2,18}$ = 0.84, p = 0.442; male: $F_{2,15}$ = 0.15, p = 0.857; sub-adult: $F_{2,11}$ = 1.23, p = 0.312; Fig. 2.4). This indicates that *A. chryspoterus* responded similarly to all three treatments and that snorkeler presence may elicit a similar initial wary behaviour to model predators or other novel objects.

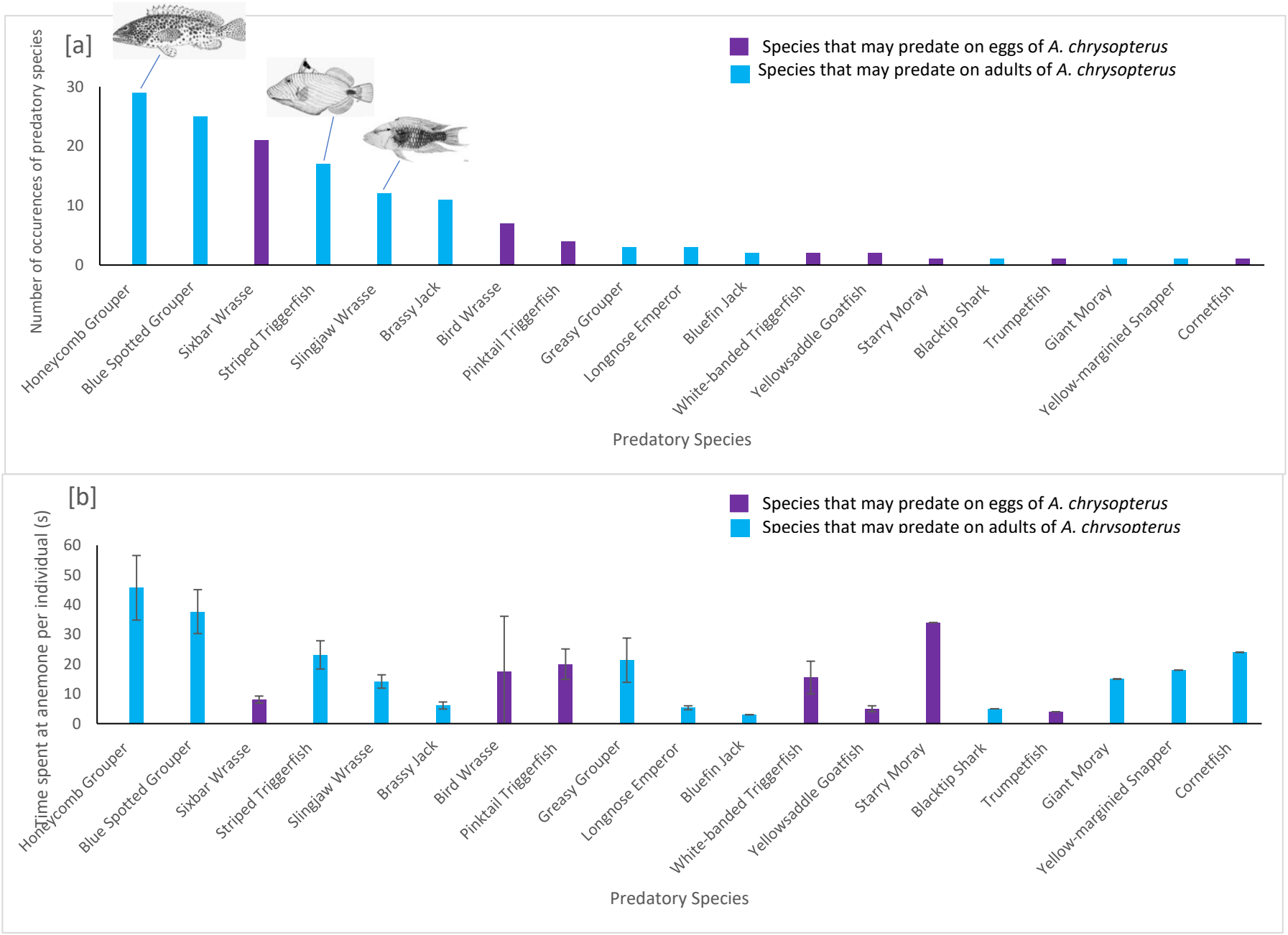



**Figure 2.3**: The predator occurrences from observational videos around six anemone sites. [a] The number of individuals detected within the camera field of view during the 30–40 min trial videos per site. [b] Mean ± SE time spent per individual within the camera field of view, within 8 m of the anemone site. Images in [a] represent a species from the three families with the highest occurrence (Serranidae, Labridae and Balistidae). Images are from the FAO and sourced from FishBase.de

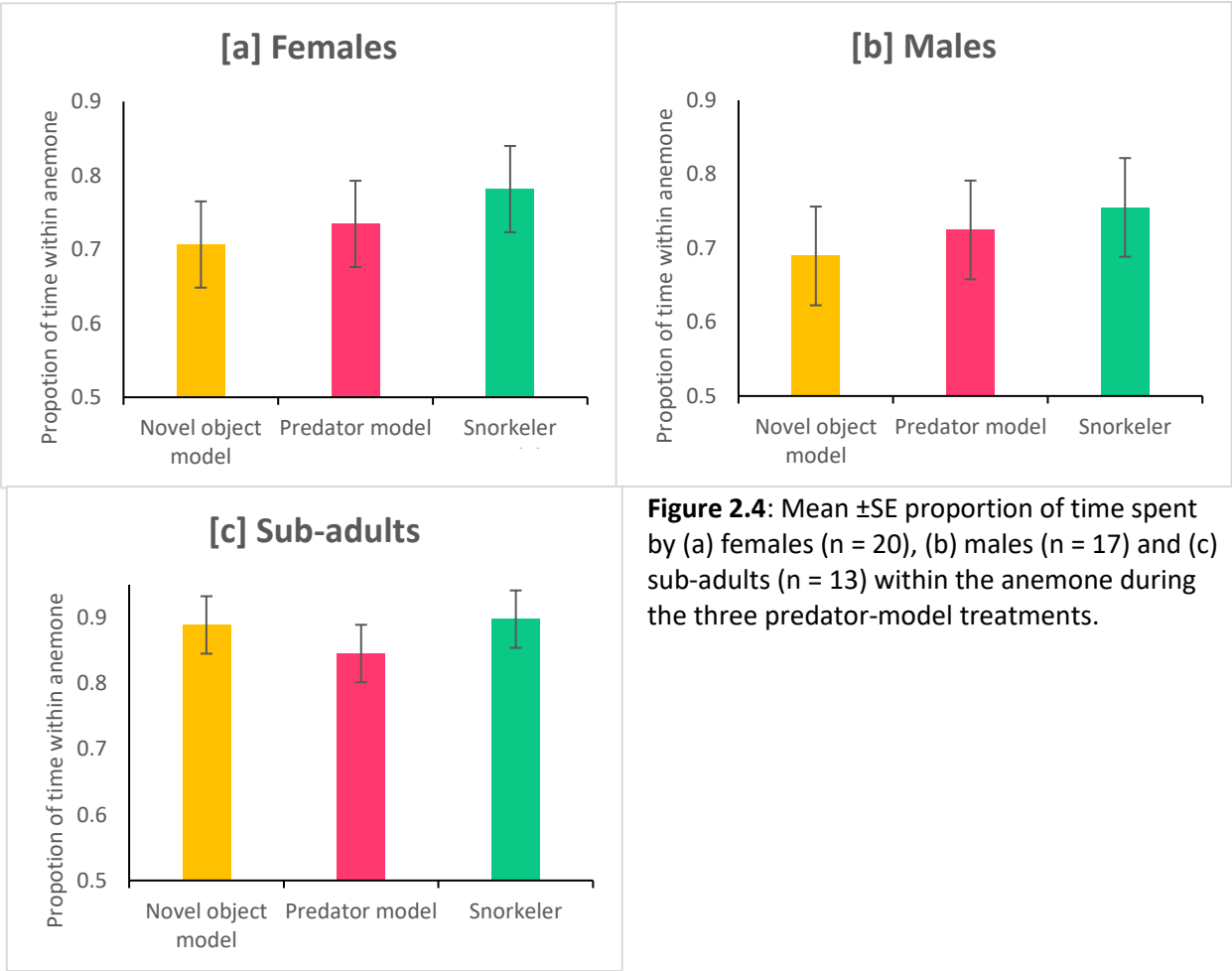



**Figure 2.4**: Mean ±SE proportion of time spent by (a) females (n = 20), (b) males (n = 17) and (c) sub-adults (n = 13) within the anemone during the three predator-model treatments.

### 2.3.2i Female behavioural responses

Evasive behaviour of females was significantly affected by treatment in terms of both rate of moving away from the model (repeated-measures ANOVA: $F_{2,18} = 15.29$, $p < 0.001$; Fig. 2.5a) and rate of abandoning the anemone ($F_{2,18} = 9.33$, $p < 0.001$; Fig. 2.5b). For both behaviours, females increased their evasive behaviour in the predator model (paired t-tests, moving away: $t_{19} = -5.07$, $p < 0.001$; abandoning: $t_{19} = -4.18$, $p < 0.001$) and novel object (moving away: $t_{19} = -4.69$, $p < 0.001$; abandoning: $t_{19} = -3.41$, $p = 0.003$) trials compared to the snorkeler control trials. There was no significant difference between predator model and novel object trials for either movements away from the model ($t_{19} = 1.73$, $p = 0.776$) or for rate of abandoning the anemone ($t_{19} = 0.55$, $p = 0.588$).

Similarly, there was a treatment-based significant difference in wary/agitated behaviour of females with respect to both time spent agitated (repeated-measures ANOVA: $F_{2,18} = 3.38$, $p = 0.045$; Fig. 2.5c) and head nodding ($F_{2,18} = 6.18$, $p = 0.005$;

Fig. 2.5d). As before, both behaviours were significantly higher in the predator model (paired t-tests, agitated: $t_{19} = -2.99$, $p = 0.007$; head nodding: $t_{19} = -3.13$, $p = 0.005$) and novel object (agitated: $t_{19} = -2.88$, $p = 0.010$; head nodding: $t_{19} = -3.08$, $p = 0.006$) trials compared to snorkeler control trials. There was no significant difference between the predator model and novel object trials for time spent agitated ($t_{19} = -0.07$, $p = 0.948$) and for time spent head nodding ($t_{19} = 0.42$, $p = 0.680$).

### 2.3.2ii Male behavioural responses

Evasive behaviour of males was significantly affected by treatment in terms of both rate of moving away (repeated-measures ANOVA: $F_{2,15} = 8.56$, $p = 0.001$; Fig.2.6a) and rate of abandoning the anemone ($F_{2,15} = 5.14$, $p = 0.012$; Fig. 2.6b). For both behaviours, males increased their evasive behaviour in the predator model (paired t-tests, moving away: $t_{16} = -5.31$, $p < 0.001$; abandoning: $t_{16} = -3.34$, $p = 0.004$) and novel object (moving away: $t_{16} = -3.01$, $p = 0.008$; abandoning: $t_{16} = -2.71$, $p = 0.015$) trials compared to the snorkeler control trials. There was a treatment-based non-significant trend in the male trial time spent agitated (repeated-measures ANOVA: $F_{2,15} = 2.78$, $p = 0.078$) in which *post hoc* analysis demonstrated a significant increase between treatments in time spent agitated when exposed to the predator model compared to the snorkeler control (paired t-test: $t_{16} = -2.60$, $p = 0.019$). There was no significant difference in the time spent agitated when exposed to the novel object compared to snorkeler control ($t_{16} = -1.43$, $p = 0.173$) or between the wood and model treatments ($t_{16} = 0.10$, $p = 0.922$). There was a strong, but statistically non-significant, treatment-based difference in the male trial time spent head nodding (repeated-measures ANOVA: $F_{2,15} = 3.23$, $p = 0.054$). *Post hoc* analysis demonstrated a non-significant trend in the increase in head nodding when exposed to the predator model compared to snorkeler control (paired t-test: $t_{16}$ $t = -1.97$, $p = 0.066$), whilst there was no significant difference in response to the novel object and the snorkeler control ($t_{16} = -1.97$, $p = 0.17$) nor to the predator model and novel object ($t_{16} = 0.49$, $p = 0.63$).

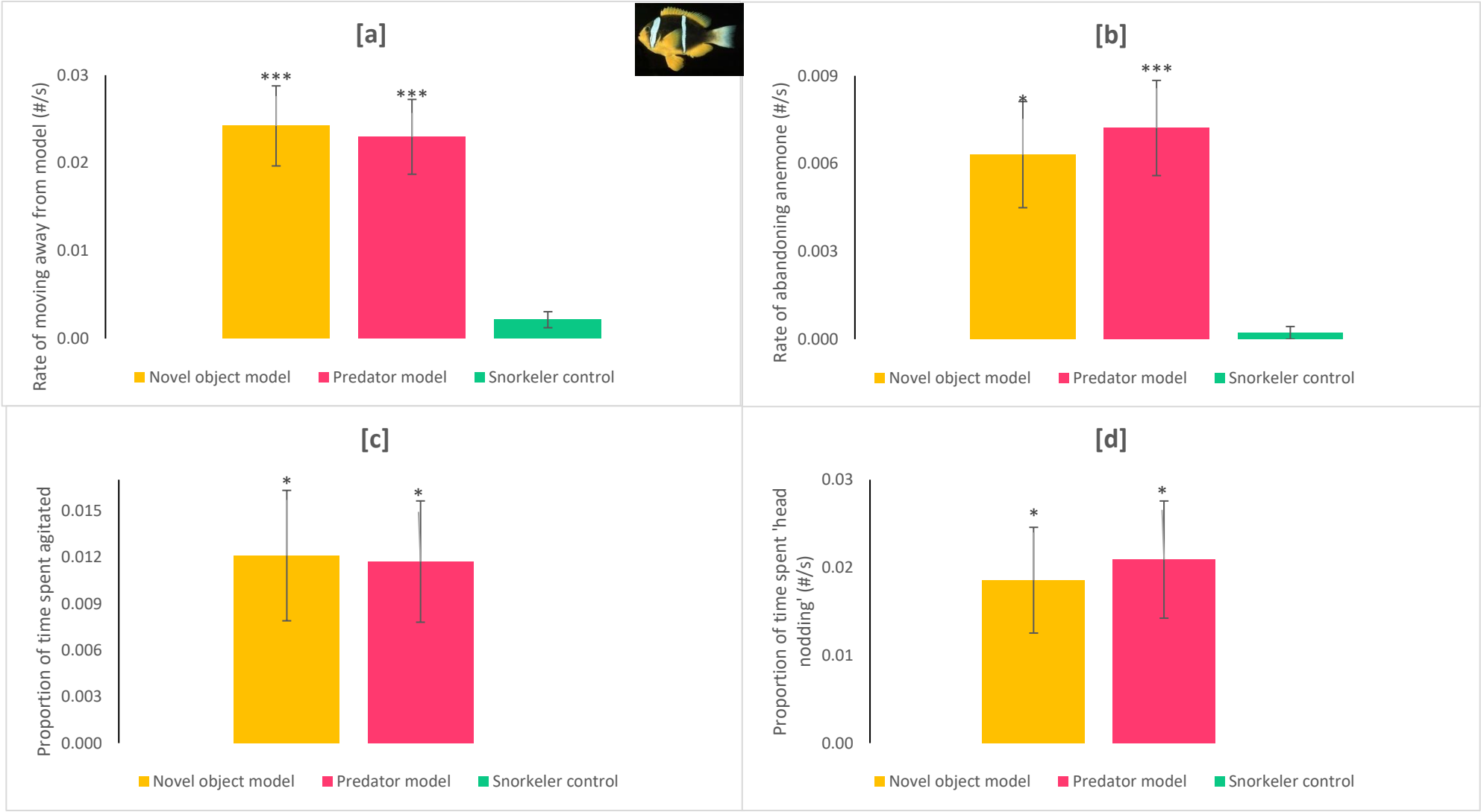



**Figure 2.5:** Female *A. chrysopterus* behavioural responses to different treatments. [a] Rate of females moving away from model during trial, [b] rate of females abandoning anemone during trial, [c] proportion of trial time females spent agitated and [d] proportion of trial time females spent 'head nodding'. Bars are mean values ± standard error. Asterisks indicate bars that are significantly different from the snorkeler control. *$p < 0.05$, ***$p < 0.001$. (Image taken by J.E. Randall (1968) sourced from FishBase.de)

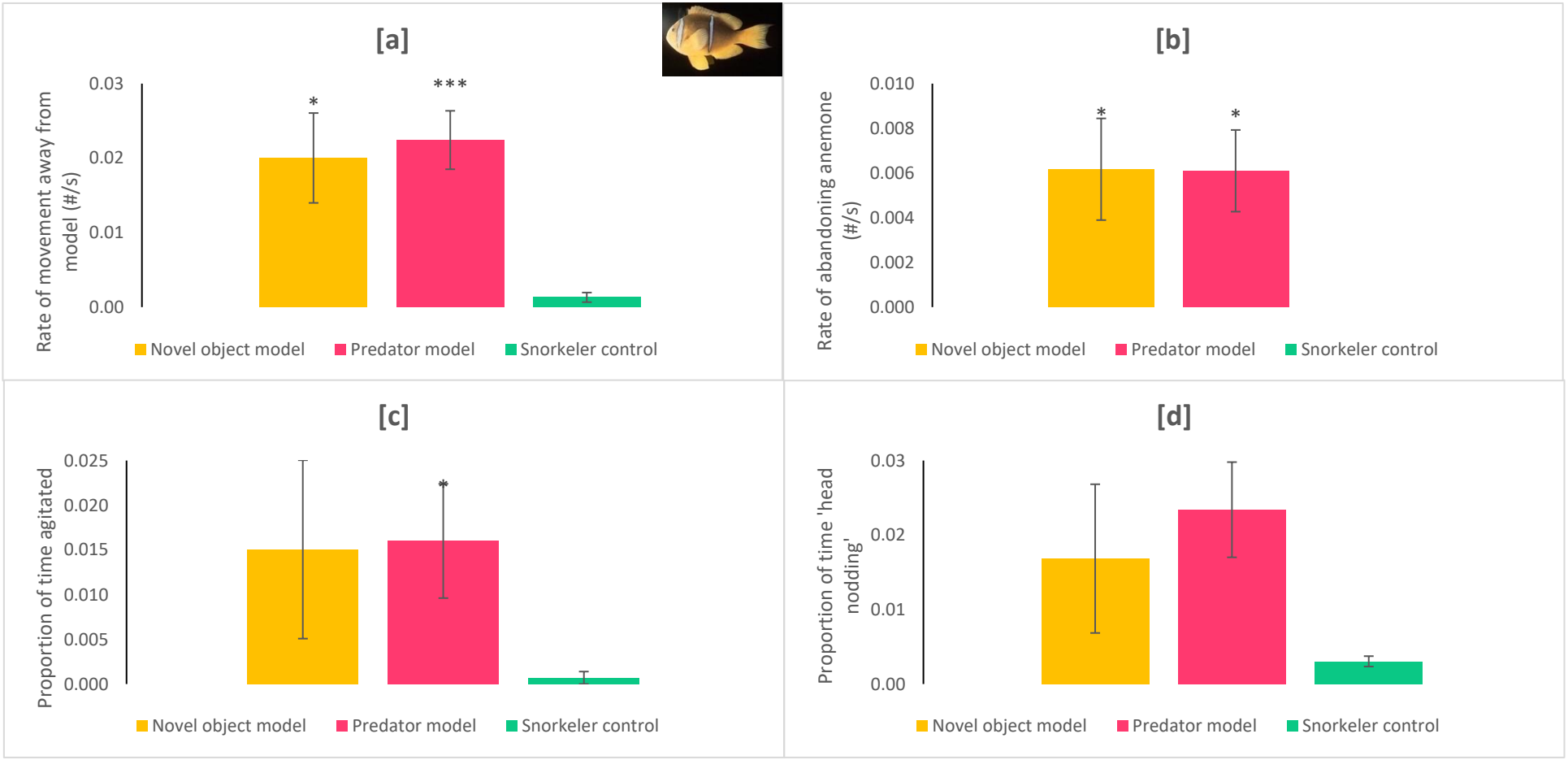



**Figure 2.6**: Male *A. chrysopterus* behavioural responses to different treatments. [a] Rate of males moving away from model during trial, [b] rate of males abandoning anemone during trial, [c] proportion of trial time males spent agitated, and [d] proportion of trial time males spent 'head nodding'. Bars are mean values ± standard error. Asterisks indicate bars that are significantly different from the snorkeler control. *$p < 0.05$, ***$p < 0.001$. (Image taken by J.E. Randall (1969) sourced from FishBase.de)

### 2.3.2iii Sub-adult behavioural responses

Sub-adult *A. chrysopterus* evasive behaviour was significantly affected by treatment in terms of moving away (repeated-measures ANOVA: $F_{2,11} = 15.0$, $p < 0.001$; Fig. 2.7a) and there was a non-significant trend in terms of abandoning the anemone ($F_{2,11} = 2.68$, $p = 0.091$; Fig. 2.7b). For both behaviours, sub-adults increased their evasive behaviour in the predator model (paired t-tests, moving away: $t_{12} = -7.58$, $p < 0.001$; abandoning: $t_{12} = -2.35$, $p = 0.037$) and novel object (moving away: $t_{12} = -4.04$, $p = 0.002$; abandoning: $t_{12} = -2.77$, $p = 0.017$) trials compared to the snorkeler control trials. There was no significant difference between predator model and novel object trials for either movements away from the model ($t_{12} = 1.53$, $p = 0.151$) or for the rate of abandoning the anemone ($t_{12} = 0.02$, $p = 0.986$). There was no significant treatment-based difference in the wary/agitated behaviour of sub-adults with respect to time spent agitated (repeated-measures ANOVA: $F_{2,11} = 2.65$, $p = 0.093$; Fig. 2.7c). There was, however, a significant difference in sub-adult head nodding behaviour ($F_{2,11} = 5.31$, $p = 0.013$; Fig. 2.7d) in which head nodding behaviour was significantly higher when exposed to the predator model (paired t-test: $t_{12} = -3.47$, $p = 0.005$) compared to the snorkeler control. Head nodding behaviour showed a non-significant trend to be higher when exposed to the novel object compared to the snorkeler control ($t_{12} = -2.07$, $p = 0.061$). There was no significant difference between predator model and novel object trials for the proportion of time spent head nodding ($t_{12} = 0.87$, $p = 0.402$).

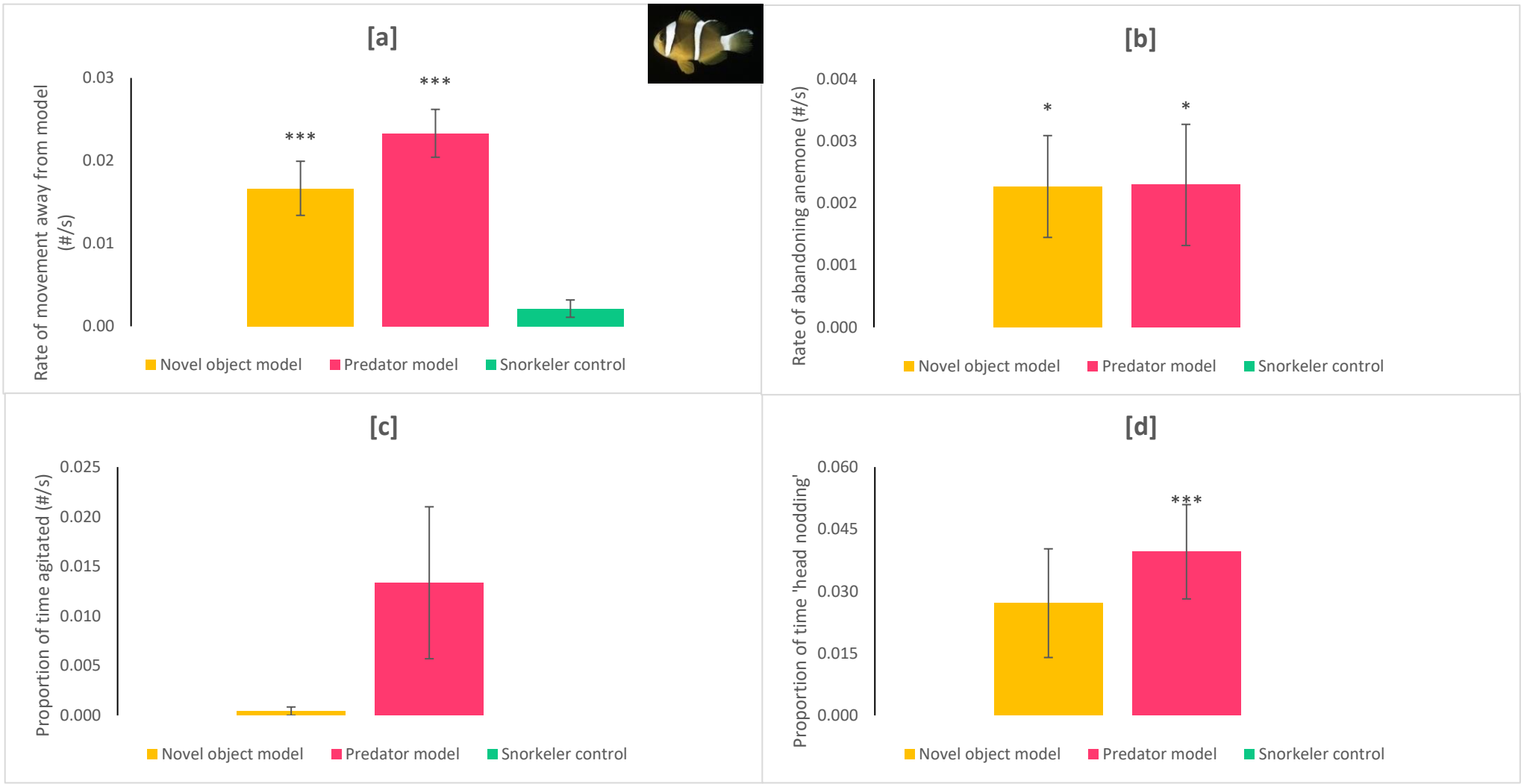



**Figure 2.7:** Sub-adult *A. chrysopterus* behavioural responses to different treatments when present. [a] Rate of sub-adults moving away from model during trial, [b] rate of sub-adults abandoning anemone during trial, [c] the proportion of trial time sub-adults spent agitated and [d] the proportion of trial time sub-adults spent 'head nodding'. Bars are mean values ± standard error. Asterisks indicate bars that are significantly different from the snorkeler control. *$p < 0.05$, ***$p \leq 0.001$.(image taken by J.E. Randall (1968) sourced from FishBase.de

### 2.3.2iv Vocalisations

The rate of vocalisations detected differed significantly across treatments (repeated-measures ANOVA: $F_{2,19} = 3.90$, $p = 0.029$; Fig. 2.8). The rate of vocalisations was significantly lower in the predator model treatment compared to both the novel object (paired t-test: $t_{19} = 2.39$, $p = 0.027$) and snorkeler control ($t_{19} = -2.62$, $p = 0.017$) treatments. There was no significant difference between the novel object and snorkeler control treatments ($t_{19} = -0.12$, $p = 0.917$).

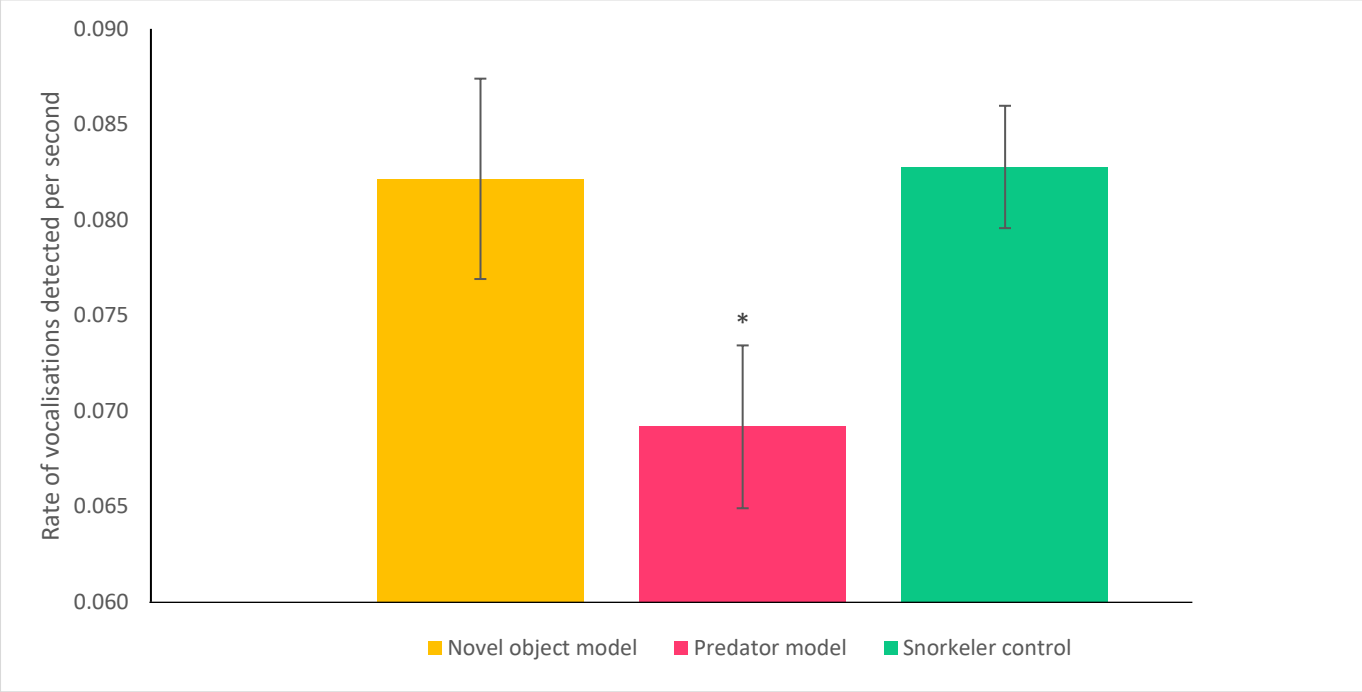
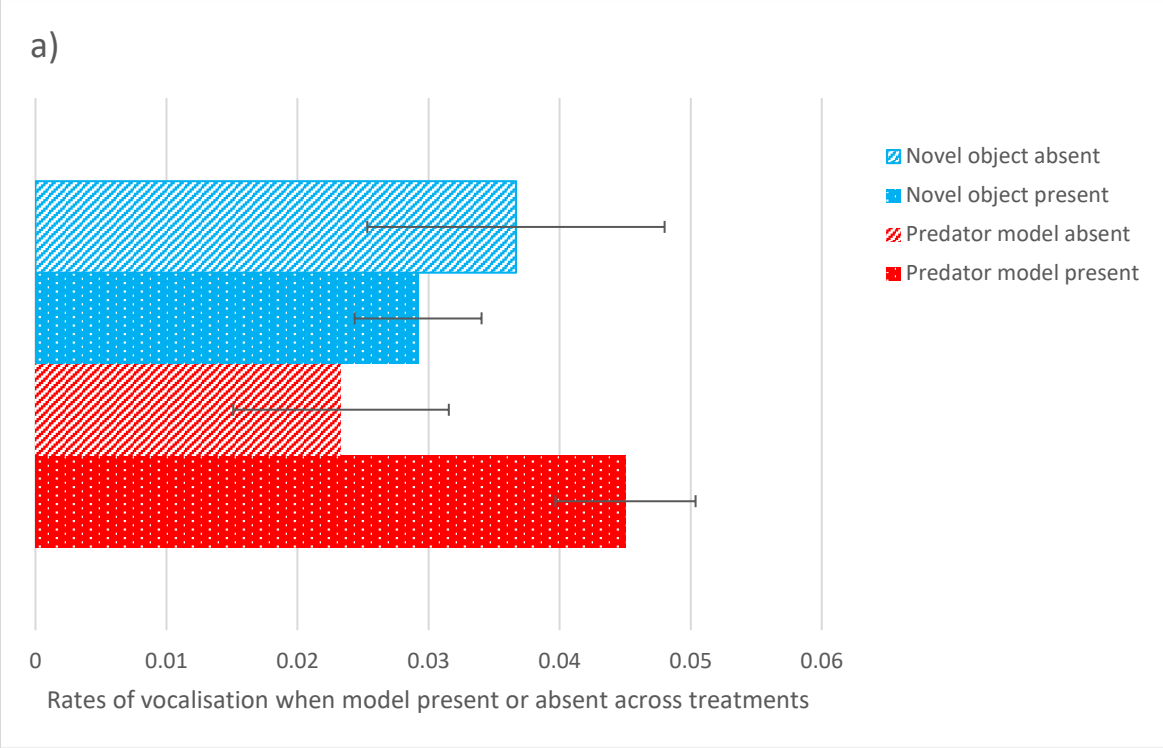



**Figure 2.8:** Rate of vocalisations detected throughout trial period across treatments. Bars are mean values ± standard error. Asterisks indicate treatment that is significantly different from other treatments, both being *p < 0.05.

There was a treatment-based non-significant trend between the predator model and novel object treatments of a higher rate of vocalisations when the predator model was present than compared to the presence of the novel object (paired t-test: $t_{19} = 2.05$, $p = 0.054$; Fig. 2.9). In 70% of the trials, fish vocalised at a higher rate when the predator model was present than compared to when exposed to the presence of the novel object at the same anemone site. In addition, in absence of the model during the trial, there was non-significant trend in the rate of vocalisations in the predator model treatment compared to that of the novel object ($t_{19} = -2.02$, $p = 0.058$). When the model was absent during the trial, 65% of fish vocalised at a higher rate when the novel object model was absent compared to when the predator model was absent during the trial.



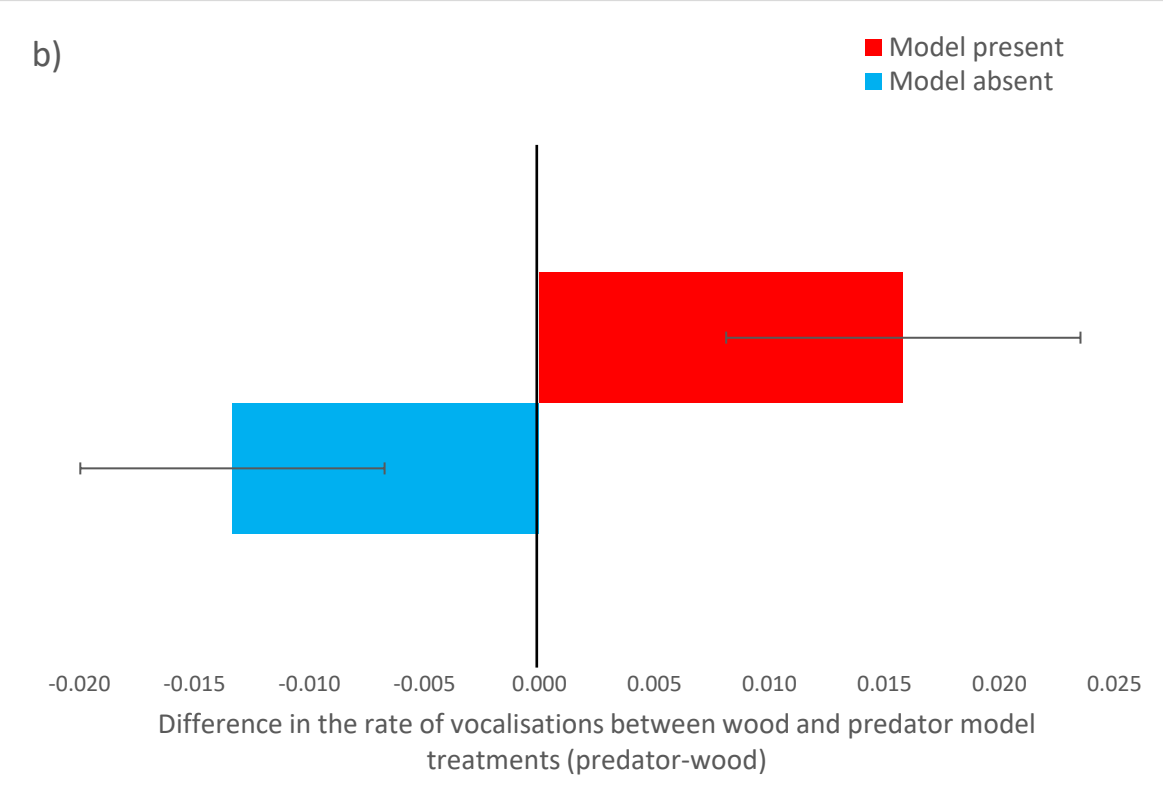




**Figure 2.9:** a) The rate of vocalisation between predator model and novel object for each site when the model was either present or absent during the trial. Bars are mean values ± standard error. b) The differences between rate of vocalisation between predator model and novel object during model presence or absence in each trial. Positive values indicate that there were more vocalisations when exposed to the predator model compared to the novel object, negative values indicate more vocalisations when exposed to the novel object than the predator model. Bars are mean values ± standard error.

### 2.3.3 Stage 3: Behavioural response to acoustic playback

#### 2.3.3i Immediate 5 s response

There was no significant difference when comparing the 5 s response to alarm and ambient playback treatments across all sites (Fig. 2.10). When comparing the change in 5 s response before and after the onset of either playback treatment per site, there was a non-significant trend (McNemar's test: n = 24, p = 0.074), with more fish responding after the onset of the alarm playback. There was no significant change in the 5 s response before and after the onset of the ambient playback (n = 24, p = 0.371).

#### 2.3.3ii Time spent outside anemone

The proportion of the 25 s trial *A. chrysopterus* spent outside the anemone (a measure of the sheltering response to acoustic stimuli) was not significantly different between alarm and ambient playback treatments for any cohort of individuals (paired t-tests, females: $t_{17} = 0.85$, p = 0.405; males: $t_{18} = -1.06$, p = 0.302; sub adults: $t_{7} = -0.42$, p = 0.685; Fig. 2.11). There was a non-significant trend in the change in response before and after the onset of playback per site in males ($t_{18} = 1.83$, p = 0.084) in which the onset of the alarm playback caused an average decrease in the proportion of time spent outside the anemone compared to during ambient playback (Fig. 2.11). Females ($t_{21} = -0.54$, p = 0.595) and sub-adults ($t_{9} = -0.62$, p = 0.549) showed no significant difference in response before and after onset of playback between alarm and playback treatments.

### 2.3.3iii Head turning behaviour

There was a non-significant trend in the difference in *A. chrysopterus* head turning behaviour (a measure of wary or vigilance behaviour in response to acoustic stimuli) within the 25 s trial before and after the onset of playback for both alarm and ambient treatments (paired t-test: $t_{46} = 1.94$, $p = 0.059$). This demonstrates that individuals tended to increase the rate of head turning after the onset of the alarm playback compared to ambient playback. When females and males were considered separately, however, there was no significant difference in the change in head turning rate before and after the onset of either playback treatment (females: $t_{20} = -9.94$, $p = 0.357$; males: $t_{15} = -0.83$, $p = 0.420$); sub-adults showed a non-significant trend in the difference in head turning rate before and after the onset of the alarm playback compared to the ambient playback treatment ($t_9 = -2.13$, $p = 0.062$).

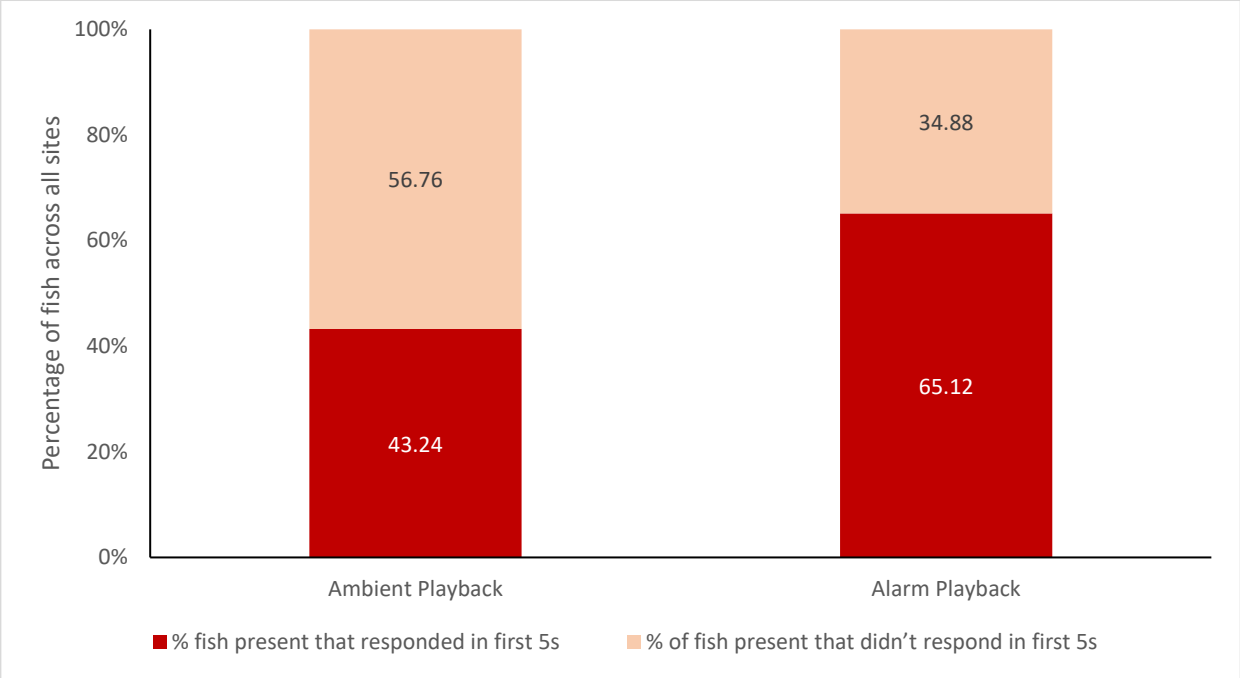



**Figure 2.10.** The percentage of fish at each anemone site that either responded or did not in the first 5 s of the playback track. Response was categorised as either swimming towards the anemone or into the anemone, no obvious behaviour change was categorised as no response. Individuals that were already in the anemone at the onset of the playback were excluded.

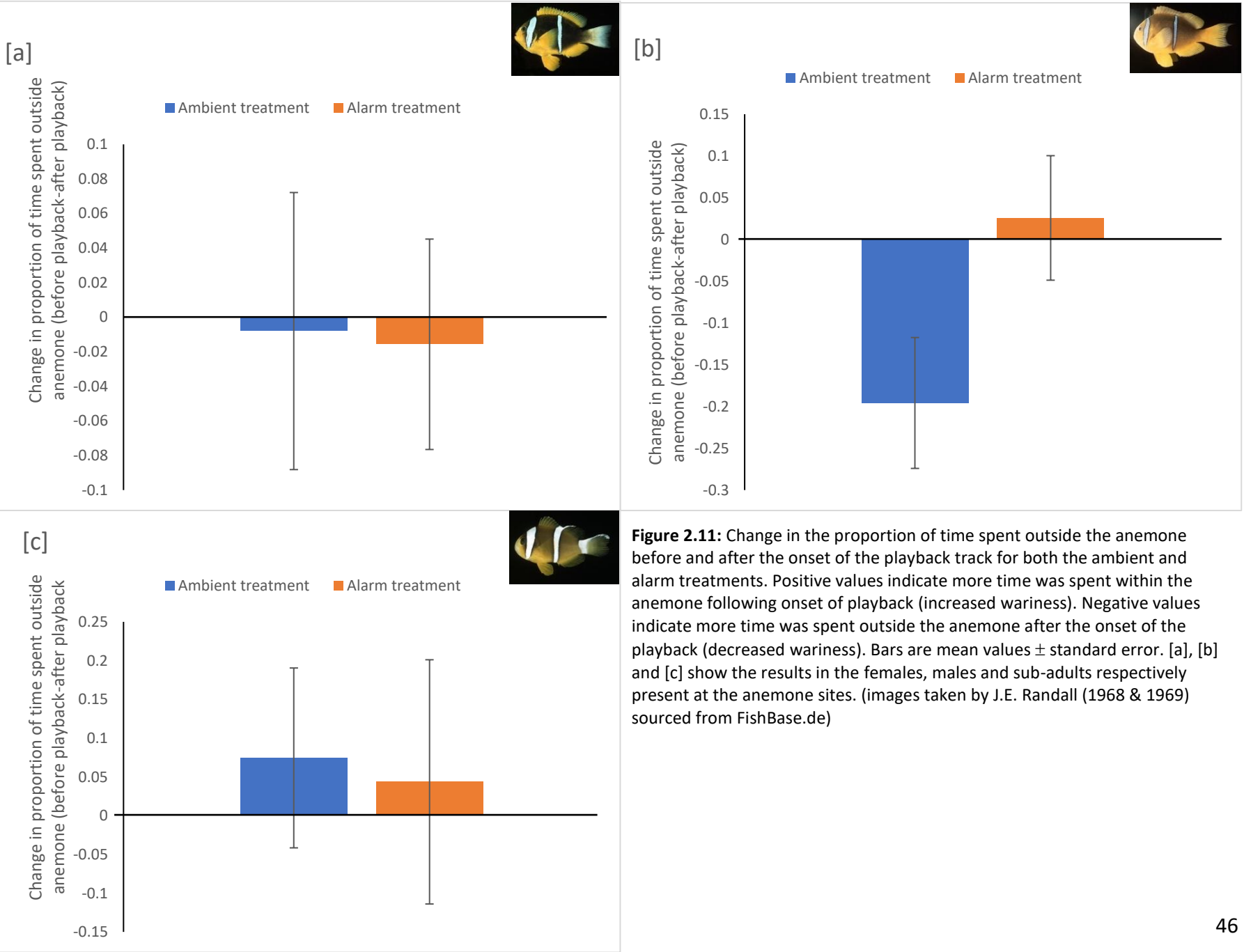



**Figure 2.11:** Change in the proportion of time spent outside the anemone before and after the onset of the playback track for both the ambient and alarm treatments. Positive values indicate more time was spent within the anemone following onset of playback (increased wariness). Negative values indicate more time was spent outside the anemone after the onset of the playback (decreased wariness). Bars are mean values ± standard error. [a], [b] and [c] show the results in the females, males and sub-adults respectively present at the anemone sites. (images taken by J.E. Randall (1968 & 1969) sourced from FishBase.de)

## 2.4 Discussion

### 2.4.1 Stage 1: Ecological context

Baseline observational videos demonstrated that grouper were the most abundant predators, spending the most time around the anemone colonies. The honeycomb grouper (*Epinephelus merra*) was the most commonly observed within this family. Although there was no conclusive evidence due to the lack of a witnessed predatory attack on clownfish, the honeycomb grouper appeared to be the most relevant species in terms of direct predation risk.

Predator observation videos were taken during the dawn period (5:00–7:00 am) as crepuscular hours experience high levels of activity (e.g. hunting) and vocalisation rates (Condini et al. 2011; Ghazali, 2011; McWilliam et al. 2017; Putland et al. 2017). Predation events are very rare to catch on camera, however, presenting a challenge in assessing the level of threat to *A. chrysopterus* by different piscivorous species. Threat level is known to affect anti-predator responses in fish (Brown et al. 2006). As many of the potential predatory species are often present near the anemones, the standardised movements of the predator model in Stage 2 incorporated jolting "attacks" on *A. chrysopterus* to intensify the threat of the model relative to presence alone.

A single predatory attack by a grouper was detected in the observational recordings near the anemone (but was not directed at the clownfish) during which all *A. chrysopterus* retreated into their anemone. Grouper feed primarily on crab and fishes, posess generalist feeding behaviours and hunt with a high degree of opportunism (Condini et al. 2011). Indeed, Vail et al. (2013) demonstrate that groupers will hunt cooperatively with moray eels and octopus when suitable opportunity arises to increase prey encounters. Other studies note grouper individuals only attacking their prey once it is distracted by an attack from another predator, sometimes totalling only three attacks across a two hour period (Neill & Cullen, 1974). This demonstrates that, although attacks are infrequent, grouper pose a "stealth and ambush" threat when present in a natural setting, and as opportunistic generalists, make a justified choice of species in the predator model for *A. chrysopterus*. In addition, multiple grouper species, according to fishermen and

academics alike, tend to position themselves below their prey and attack upwards (Neill & Cullen, 1974). This suggests that the standardised jolting movements of the predator model in the “puppet” system would more effectively replicate a grouper hunting strategy than the pulley system.

### 2.4.2 Stage 2: Call usage and call production

All life stages of *A. chrysopterus* spent similar amounts of time within the anemone when exposed to the predator model, novel object and snorkeler alone. Females, males and sub-adults initiated significantly more evasive behaviour (moving away from the model and abandoning anemone) when exposed to the predator model and novel object compared to the snorkeler control (near significant difference abandoning the anemone in sub-adults). Females and males demonstrated significant increases in wary/agitated behaviours in terms of time spent agitated when exposed to the predator model. Females and sub-adults demonstrated significant increases in wary/agitated behaviour through head nodding when exposed to the predator model, whilst males only demonstrated a non-significant trend in increased head nodding when exposed to the predator model.

During an attack by a predator, the clownfish would be expected to spend more time in an anemone where it is protected from predators, than when no predator was present (Feeney & Brooker, 2017; Schmiege et al. 2017). In this mutualistic relationship, clownfish protect the anemone from predators (like turtles) whilst sheltering from their own predators, defended by the stinging nematocysts in the anemone tentacles, to which they are protected by a mucus coating (Brooks & Mariscal, 1984; Schmiege et al. 2017). A female clownfish lives almost six times longer when associated with an anemone compared to a similarly sized female without an anemone (Feeney & Brooker, 2017). Despite this protection, in this study *A. chrysopterus* did not significantly differ in the time spent within the anemone between the three treatments of predator model, novel object or snorkeler presence. Wariness of snorkeler presence could partly explain this, as observer presence can alter the acclimation time required for fish to return to pre-disturbance behaviours (Nanninga et al. 2017). Individuals could therefore remain within the anemone in response to predation risk for a recognised predator (the predator model), but also

express neophobia (a generalised avoidance response to unknown stimuli) towards the model object and snorkeler, thus masking any observable effects (Brown et al. 2013).

A proportion of each trial included time in which the model was not actively attacking. This is because time had been incorporated into the standardised movements used in which the model and/or snorkeler was away from the anemone. It is therefore possible that any initial anti-predator response metrics such as retreating into the anemone would be diluted throughout the trial, perhaps due to the need for fish to balance the trade-off between assessing predation risk and other fitness-related activities (such as foraging and resource defence) (Brown, 2003). Individuals may only briefly respond with anti-predator behaviours at appropriate moments, thereby maximising time available for other activities and increasing their fitness potential (Brown, 2003). This could especially be the case with “resident predators” such as the predatory species of grouper selected in this study, *Epinephelus merra*, that are often present in the area.

In addition to time spent in the anemone, other behavioural measures enabled the examination of responses to a sustained predatory presence and threat. Across all three life stages (female, male and sub-adult), *A. chrysopterus* demonstrated evasive and/or agitated behaviours in response to the predator model and novel object throughout the trial. This could signify that despite low level wariness towards snorkeler presence, individuals demonstrated higher levels of anti-predator responses to threats posing a greater predation risk. In environments dominated by opportunist predators and where predation risk is rarely predictable across time and space, fish must constantly adapt costly anti-predator responses (Brown et al. 2013). The stark difference in both evasive and agitated responses towards snorkeler presence alone and to the other treatments demonstrates that the novel object and predator model were consistently perceived as a greater threat. With few differences between predator model and novel object, however, the threats of a novel object and a recognised predator appear to be equally prioritised in the anti-predator responses of *A. chrysopterus.* Neophobia appears to influence a fish’s evasive and agitated responses similarly to when exposed to a predator. An alternative possibility however is that both predator model and novel object produced neophobic evasive

reactions in the fish studied. The use of a non-predatory fish model would elucidate whether fish could in fact recognise the predator model as a predator and distinguish it from other novel objects, responding appropriately to the potential threat of either.

It is important to note that different individuals will have varying prior experience of exposure to snorkeler presence as well as to predation levels. Anemone sites vary in proximity to touristic destinations and different sites are found in areas either open or restricted to fishing for species such as grouper, affecting the ecology of that area and the exposure levels to different stimuli. This baseline experience informs behavioural responses, as prey species rely on previous experience and level of risk to tailor and perform their adaptive anti-predator responses (Brown et al. 2013, 2018). This could, therefore, introduce more variation in the types and levels of responses seen during this study.

Sub-adult *A. chrysopterus* spent comparatively more time in the anemones than both the dominant female and male. As the smallest individuals in the colonies, sub-adults are vulnerable to a wider range of predators and size range of predatory species, remaining outside the gape-size refuge offered to larger individuals (Scharf et al. 2000; Persson et al. 2018). The heightened vulnerability, compounded by factors such as lower swimming performance in smaller individuals, can influence prey escape responses (Scharf et al. 2000). Smaller individuals are more protected within the anemone tentacles and may retreat more readily than their larger, dominant counterparts. This would explain why sub-adults spent more time in the anemones and were 3–3.5 times less likely to abandon the anemone under threat during the model exposure. The larger individuals may not, in turn, be sufficiently covered by the anemone tentacles and therefore prefer further evasion by abandoning the anemone, rather than depending on the nematocyst sting as a predatory deterrent.

Sub-adults spent, on average, almost twice as much time “head-nodding” when exposed to the predator model than the other life stages. This behaviour possibly acts as a form of communication with other members of the group. Clownfish species are known to conduct movements including elevation of the head that are associated with, but not isolated to, sound production and communication

(Parmentier et al. 2007; Colleye & Parmentier, 2012). Clownfish acoustics and behavioural responses have, thus far, been broadly categorised into antagonistic and reproductive sounds (Parmentier et al. 2007; Colleye et al. 2009, 2011; Colleye & Parmentier, 2012). These studies show that individuals of different life stages vocalise and respond differently to others, but thus far, they do not describe such responses in the context of predation. As head nodding behaviour was often associated with the approach of the predator model, often followed by evasive responses, it is possible that this behaviour provides a primary form of communication of wariness to a potential predator threat. As sub-adult clownfish conduct “quivering” behaviours as part of the submissive posturing in antagonistic interactions in the size-based dominance hierarchy, it is possible that body movements communicate signals in other ecological contexts as well (Colleye et al. 2009). As small individuals are more sensitive to the lower frequencies produced in vocalisations by larger conspecifics, it is possible that sub-adults communicate wariness to dominants, responding to vocalising dominants when threatened (Colleye & Parmentier, 2012). Across all individuals, head nodding could act as a less costly initial signal of a potential threat prior to more costly evasive tactics, such as vocalising (which is energetically costly and may attract further attention), or abandoning the anemone (which is a crucial resource and the primary line of defence). Smaller individuals, vulnerable to a wider range of predators, are protected within the anemone and may utilise this form of communication more often. This would be in keeping with the threat-sensitive predator avoidance hypothesis in which prey match the intensity of their anti-predator responses to the level of threat posed by the predator (Brown et al. 2006). An approaching predator model may not yet trigger the highest level of urgency and thus demand a less costly initial form of communication, resorting to alarm calling if the threat level and urgency were to increase (Magrath et al. 2015). Indeed, Helfman (1989) demonstrated that prey fish can exhibit progressively stronger anti-predator responses with increasing threat level of a predator.

Total vocal activity during the trial incorporates vocalisations both relevant and unconnected to the predator model. Clownfish are known to produce chirps, grunts and pops including dominant and submissive sounds during antagonistic interactions (Parmentier et al. 2007; Colleye & Parmentier, 2012). Clownfish usually occupy a

strict size-dominance hierarchy, where size relates directly to ranking in the queue to dominant status and where smaller newer recruits are only promoted in rank when a higher rank individual leaves or perishes (Colleye et al. 2011; Colleye & Parmentier, 2012). As vocalisations are honest representations of size in clownfish (pulse duration and dominant frequency are determined by size), vocalisations form a common and important component to antagonistic interactions that maintain size differences and thus ranking and dominance (Colleye et al. 2009). Vocalisations recorded over a trial will likely have a wide range of ecological functions. It is interesting, therefore, that the overall rate of vocalisations significantly decreased in the presence of the predator model compared to the presence of the snorkeler and the novel object. Many fish demonstrate a “holding quiet” behaviour, a reduction in the level of vocalising when threatened in order to reduce attracting an eavesdropping predator (Myrberg, 1981; Lobel et al. 2010). Indeed, delivering louder calls or vocalising at a higher rate can be innately more risky in attracting predators (Horn & Leonard, 2008). It is therefore possible that *A. chrysopterus* reduce their antagonistic and/or reproductive vocalisations when threatened by predator presence. This would explain the significant reduction in the rate of vocalisations detected when fish were exposed to a predator.

Having only one hydrophone at a site recording any acoustic behaviour precluded identifying the signalling fish. One can use behavioural measures in addition to vocalisations as a means of judging which individual has vocalised—for example, Parmentier et al. (2007) used the movements of mouth parts to discern which individual vocalised—but this is difficult to confirm in the field where individual fish may not always be in close view. In the current study, sound sources could not be pinpointed and could not therefore be used to assign a vocalisation to a particular individual and their associated behaviour. Asssessing whether the vocalisations ocurred during model presence or absense increased confidence in which of the vocalisations detected were produced in relation to predatory threat. The current study found that the rate of vocalisations was higher during predator model presence than during presence of the novel object. It also found the rate of vocalisations was higher during temporary absence of the wood model than absence of the predator model. Predator presence, a threat induced by the predator model, may restrict vocalisations to those that are important enough to risk being conspicuous, including

those directly related to the predator presence. Although one might expect there to be no significant difference in the rate of vocalisation when either predator model or novel object were absent, the fact there was a trend of a lower rate of vocalising when the predator model was absent following an attack compared to absence of the wood model could be due to carryover effects of a higher threat level in the presence of a recognised predator compared to a novel object. During predator absence, fish may hold back their non-predator related vocalisations due to continued wariness and vigilance. The “non-consumptive effects” of predators (such as increased rate of movement, displacement and/or vigilance) can extend beyond the time in which the predator is actively present (Middleton et al. 2013). “Holding quiet” could correspond to continued evasive or agitated behaviours as well as prolonged wariness. Individuals may only produce vocalisations that communicate something directly relevant to the predator presence (e.g. alarm calls) and this “holding quiet” behaviour could carry over into the time in which the predator is absent. Indeed, silver perch (*Bairdiella chrysoura*) are known to supress their choruses and calling when detecting a predator (Radford et al. 2014). In turn, watching out for predators and responding is energetically costly and reduces time available for other fitness-related activites (such as feeding/foraging). Individuals may return more quickly to ambient behaviours and sound production levels with the novel object, given that it is not associated in this study with the same level of predation risk as *E. merra,* a predator likely recognised through learnt past experience (Brown 2003; Brown et al. 2006; Chivers et al. 2014). Carryover effects can affect an individual’s behavioural responses in a new situation and its personality can contribute to its flexibility between different responses such as latency to return to baseline behaivour post-threat (Van Oers et al. 2005).

### 2.4.3 Stage 3: Call response

*A. chrysopterus* appeared to show a trend in retreat response (in the first 5 s) to the onset of alarm call playbacks when compared with their behaviour just prior to the start of the alarm call playback. During 25 s of alarm call playback, the males demonstrated a non-significant trend in spending less time outside their anemone colony compared with prior to playback onset. All individuals when considered

together showed a non-significant trend in increased head turning behaviour on the onset of 25 s of alarm call playback compared to immediately prior to the playback.

Since *A. chrysopterus* that occupy an anemone are unlikely to be genetically related (all having settled after a pelagic life stage), and even if self-recruiting back to natal habitat may actively avoid settling at the parental colony (Jones et al. 2005), it is unlikely that the main reason for alarm calling would be inclusive fitness. Predation risk is known to have strong effects on group living species, being lower for individuals in a group and thereby reducing the likelihood of subordinates leaving to breed (Heg et al. 2004). A female and dominant male might benefit from the presence of sub-adults that contribute to anti-predator defence as well as being a mating "plan B" should the male or female perish (Heg, et al., 2004). In turn, sub-adults benefit from the 'safe-haven' provided by the group, queuing for the breeding position in the group or waiting for a low-risk nearby anemone territory to become available (Kokko & Ekman, 2002; Heg et al. 2004).

Similar to the predator model trials in Stage 2, in Stage 3 (responding to playback of an alarm call) fish also have to balance the interplay between maintaining anti-predator responses such as increased vigilance or agitation, whilst maximising other fitness related activities (Brown, 2003). Responses to playback were therefore only analysed for immediate responses to the 5 s of playback in addition to behavioural responses of the maximum alarm playback duration of 25 s. Although the 2 min analysis period may have been useful in assessing post-alarm call behaviour, it is likely that obvious anti-predator responses remain detectable for only a short period of time, especially as any anti-predator responses have no visual or olfactory stimulus as confirmation of threat. The trend in *A. chrysopterus* responding (retreating into/towards the anemone) to the 5 s of alarm playback compared to just prior indicated that clownfish appear to respond to the acoustic predator warning in isolation of other stimuli.

There was a large degree of variation in the responses of different individuals of the same species, which can be seen when comparing the average difference between treatments (alarm vs ambient playback). Comparison of responses before/after playback was selected as a measure of response since it compared differences

across treatments per individual. This comparison (before/after playback) incorporates the consistent individual differences in behaviour, or 'personality' (traits that are often genetically and phenotypically correlated) of individuals that is important to consider as there are many studies that suggest that clownfish, and reef fish more generally, have distinct personalities (Van Oers et al. 2005; Sprenger et al. 2012; Schmiege et al. 2017). Personality is likely to influence the behavioural response to a predator model and the response to an acoustic stimulus of an alarm call. Although individual response analysis before/after doesn't completely account for or incorporate personality, it is a step towards controlling for it. Personality traits can remain consistent through extreme shifts in life stage (such as sex changes in hermaphroditic fish) and influence interspecific interactions (such as the clownfish mutualism with their anemone), demonstrating its importance in the ecology of a study species (Sprenger et al. 2012; Schmiege et al. 2017). The scope of behavioural responses could vary between life stages (females, males and sub-adults) as well as anemone sites. It is especially important to consider within interactive groups, in which a male might (for example) be influenced by the response of the dominant female within the same anemone (McDonald et al. 2016; Schmiege et al. 2017). In this study, response to alarm call did differ between life stages, in that individual male *A. chrystopterus* tended to spend less time outside the anemone during the 25 s alarm playback compared to 25 s prior to the playback, whereas females and sub-adults demonstrated no such response. While dominant males may play a specific role in the size-dominance hierarchy of clownfish, one would expect all size/age classes to respond to alarm calls pertaining a sufficient threat level, irrespective of variations in personality, due to the direct consequences of predator evasion (or anti-predator responses) on survivorship (Colleye et al. 2009; Hollén & Radford, 2009). It is possible that hearing sensitivity might play a role in behavioural responses to playback of alarm calls, especially as size affects hearing abilities in clownfish (Colleye & Parmentier, 2012). Larger confamilial *Abudefduf saxatilis* individuals have been found more likely to respond to higher frequencies (1000–1600 Hz) despite all sizes being most sensitive to lower frequencies (100–400 Hz). Smaller clownfish individuals are more sensitive to a lower frequency range (100–450 Hz) produced by larger clownfish (Colleye et al. 2011; Colleye & Parmentier, 2012). Perhaps individual sensitivity to the playback and resulting behavioural response remains intimately linked to size and therefore position within

the social hierarchy. This would require further consideration beyond standardising speaker playback levels.

Clownfish are known to exhibit “posturing” behavioural responses in specific contexts (Colleye et al. 2009), yet no studies have yet categorised clownfish anti-predator responses, nor fish responses to alarm calls. Head turning behaviour, like head nodding behaviour measured in Stage 2, is considered a wary, vigilant and/or agitated behavioural response to a predatory threat. Other studies demonstrate typical anti-predator responses in fish species such as *Asterropteryx semipunctatus* and *Amatitlania nigrofasciata*, including decreased feeding strikes, decreased movements and increased bobbing or posturing behaviour in response to predator stimuli (Brown et al. 2006, 2013; McCormick & Manassa, 2008). As there was a near significant trend of increased head nodding behaviour in alarm call playback compared to ambient conditions across all individuals, it would be expected that all life stages (irrespective of personality or social ranking) increase wary behaviours to avoid predation. Certain predator stimuli must be of an intensity or indicating a sufficient level of threat to pass the “minimum behaviour response threshold”. If the playback of acoustic alarm calls was near the margins of this threshold, it would increase the reliance on visual risk assessment and/or novel predator cues (Brown et al. 2006). This gradation of behavioural responses might, in part, explain the near significant trends across behavioural measures and life stages of *A. chrystopterus* to isolated alarm calls. Although alarm calls were found to elicit appropriate wariness and anti-predator responses in certain age/sex classes, perhaps alarm calls elicit anti-predator responses but individuals require further stimuli to continue such anti-predator behaviours.

This study does not directly provide conclusive evidence of alarm calling in *A. chrysopterus* in which individuals rely solely on acoustic stimuli to elicit particular anti-predator behavioural responses irrespective of life-stage, social ranking and personality. Although alarm calls may have been produced, they may also have been more general “disturbance” vocalisations that require further, perhaps multi-sensory, stimuli to reliably elicit anti-predator responses. There is, however, evidence of a vocal “holding quiet” behaviour in addition to anti-predator behaviours in association with the appearance of a predator and novel object.

Understanding the complex context within which fish assess and process information about the world around them, including acoustic information and formulate appropriate responses, requires further investigation. The ecological context, individual age/sex class and personalities of the fish all influence their behaviours. Such research will deepen our understanding of the role of acoustic communication under predation risk and the ability of fish to learn, respond and adapt to a changing environment. It will also help understand how changes to their environments could influence how fish communicate and thus their ability to survive.

# 3. General Discussion

All life stages of *A. chrysopterus* demonstrated some increase in evasive and/or agitated behaviour when exposed to the predator model of the honeycomb grouper. When exposed to playback tracks of alarm calls, individuals showed trends in response across the 5 s and 25 s of alarm call onset either through less time spent outside the anemone or increased vigilant head turning behaviour. These behavioural responses varied between individuals, however, and could potentially be attributed to factors including personality, previous experience to predators and life stage.

## 3.1 Improvements and challenges

### 3.1.1 Response to alarm call

The behavioural measures used in this study to measure the responses to predator presence and alarm calls (Stages 2 and 3) have not previously been used in clownfish and were selected from the baseline observational videos and trials with the predator model. As clownfish have an unusual life history, it was a challenge to select which behavioural traits are the most salient for this particular species. Clownfish remain in social interactive groups but do not move as freely as schooling fish (Ioannou et al. 2017), nor are they independent, for example defending a territory to temporarily attract a transient conspecific (Shulman, 1985). Recent studies are only beginning to translate acoustic communication in fish and its linked behavioural responses (Colleye & Parmentier, 2012). Following the approach of the

majority of recent studies of clownfish, future work on clownfish anti-predator responses to other predator stimuli could be studied in the laboratory (exposure to a real predator, predator odour, chemical alarm cues from conspecifics, or any predator vocalisations). This could enable the consistency of behavioural measures and their sensitivity to be studied further for application in the field (Myrberg, 1981; Brown, 2003; Brown et al. 2006; Chivers et al. 2014). Although far more ecologically relevant, studies in the field are challenging, in part due to many uncontrolled variables. In this case, however, it was necessary to study the communication of this species in the field, under ecologically relevant conditions, because the baseline of natural behaviours has yet to be described. It is important to assess the relevance of alarm calling and the different behavioural responses, and to "test the waters" in as natural a setting as possible.

Baseline levels of predator risk and previous experiences influence anti-predator responses (Brown et al. 2006, 2013). Changing the background level of risk, even for a few days, is sufficient to influence the intensity of anti-predator response of prey to a given stimulus (Brown et al. 2006). This introduces an additional reason why there might be variation between individuals that cannot easily be controlled for in natural field settings. The use of chemical alarm cues by Brown et al. (2006) showed a reduced intensity in anti-predator responses when fish had a high level of background risk. The repeated-measures design in this study with novel object and snorkeler helped control for sensitivity and gradation of response to the predator model.

Alarm tracks were 10 min in duration instead of 30 min to avoid habituation to the acoustic stimulus of the alarm call without the reinforcing physical presence of a predatory threat. This is important to consider as reef fish have the capacity to become habituated to extended acoustic stimulus, and "crying wolf" may dilute behavioural responses (Wheeler, 2009; Nedelec et al. 2016). Although alarm calls are only a maximum of 25 s long, it is likely that if *A. chrysopterus* are to respond it will be immediate, making 10 min a suitable combination of an acclimatisation period needed for clownfish to settle to the equipment and respond to the alarm playback (Nanninga et al. 2017).

### 3.1.2 Head turning metric

Anti-predator vigilance (scanning for predators) through focal sampling (observing an individual for a specific amount of time) has been noted in studies across many taxa and is often associated with alertness (e.g. not feeding) and head moving behaviour (Fernández-Juricic et al. 2011; Hirschler et al. 2016). Head movements enable vigilance behaviour through increased visual coverage and bringing features of the environment into focus in the most sensitive parts of the eye (Fernández-Juricic, et al., 2011). It was not possible to establish gaze direction of *A. chrysopterus*, but a near significant increase in head nodding after the onset of alarm call playback provides confidence in this behavioural measure. Factors such as sex, dominance, predation risk and resource availability affect vigilance and thus high levels of variation are to be expected between individuals (Fernández-Juricic, et al., 2011). It is important to note, however, that video footage captured occasions where larger *A. chrysopterus* fed from the anemone excretions during the trial leading to head movements. Head movements were therefore excluded when individuals conducted such anemone feeding behaviour. It is also possible that head movements would be affected by active feeding in the water column, where individuals move towards food particles in the water column. Distinguishing between feeding and vigilance behaviour was not possible from the video footage, but this potential source of error in measuring was consistent between treatments. In addition, trends in this behavioural measure were still apparent despite the possibility that the rate of head movements in ambient treatments were overestimated due to the inclusion of feeding movements when individuals are not under predation risk.

### 3.1.3 Source of vocalisations

The use of a single hydrophone per site in this study meant sound sources could not be isolated by triangulation. Alternatives such as a hydrophone array would need to have the capacity to distinguish across small distances (clownfish often being a few centimetres apart within the anemone). Pulse duration and dominant frequency are the most important variables to measure to enable individual identification and are related to the size of the fish (Parmentier et al. 2009; Colleye & Parmentier, 2012). Calculating these metrics for any vocalisations produced and the alarm calls used in the playback would, therefore, enable more informed assignments as to which individual, often distinguishable by size, vocalised, even between conspecifics

(Colleye et al. 2009). This would also contribute to understanding which individual, and its relative position in the size-dominance hierarchy, is most sensitive to the alarm call playbacks. This is because the greatest hearing sensitivity of individuals is not necessarily the dominant frequency of the sounds they emit when vocalising. For example, sub-adult clownfish were found to be most sensitive to the dominant frequency of the calls of larger individuals (Parmentier et al. 2009; Colleye & Parmentier, 2012). Being able to identify which individual is alarm calling would enable further study of the consistency of acoustic anti-predator response across all life stages, or whether there are “socially ranked sentinels”, with particular positions in the social hierarchy tasked with informing the rest of the group of predator threats, as seen in other taxa (Kern & Radford, 2013). As almost all anemone sites had both *A. chrysopterus* and *D. trimaculatus* co-inhabiting the sea anemones, pulse period could be used to distinguish vocalisations between species, as no overlap has been found in pulse period between these species in previous studies (Colleye et al. 2011). Interspecific acoustic diversification could ensure organisms avoid the masking of important acoustic communication when calls overlap in time and space (Radford et al. 2014; Stanley et al. 2017).

## 3.2 Future avenues

### 3.2.1 Interspecific interactions

Clownfish are closely associated with their anemone, having evolved a tightly coupled mutualistic interaction (Schmiege et al. 2017). Other species, such as *D. trimaculatus* are often associated with the same anemone territories (though are not dependent on it), occupying similar acoustic spaces as *A. chrysopterus.* Throughout this study, video footage demonstrated multiple examples of interspecific interactions and/or responses to acoustic playbacks. As these species are likely to be vigilant for similar predators, mixed species schools of fish (commonly found in the natural environment) could provide benefits, such as protection against predation despite potentially increased intraspecific competition, as seen in many studies of multi-species bird flocks (Montgomery, 1981; Ward et al. 2002). Eavesdropping on the alarm calls of another species could, if it was an honest signal, increase the capacity to respond to a predator threat without having to be independently vigilant and, by providing more predator information, form an additional adaptive benefit of mixed

species aggregations (Griffin et al. 2005; Goodale & Kotagama, 2008). Exploring interspecific behavioural responses to alarm calls, and whether multiple species are the intended receivers of the acoustic signal (communication and cooperation) or not (eavesdropping) would be a worthwhile avenue into understanding the acoustic interactions and their relative importance to species occupying overlapping ecological niches.

### 3.2.2 Acoustic recording

Underwater, sound waves travel both as planar pressure waves and as laminar flow of the particles themselves (Nedelec et al. 2016). Particle motion is the propagation of the vibratory energy of the particles to their neighbours, whilst sound pressure is the compression and rarefaction of particles as the sound wave propagates (Nedelec et al. 2016). Marine organisms, including fish, are known to be sensitive to both particle motion and sound pressure, particle motion being detectable by all known fish species (Radford et al. 2014; Nedelec et al. 2016). This means that while humans can solely detect sound pressure, pressure alone (measured by hydrophones) forms an incomplete analysis of the acoustic environment of marine organisms. As instruments become increasingly accessible and affordable, studies should try to incorporate particle motion into their research (Popper & Hawkins, 2018). As particle motion is what is responded to by fish in most instances, including it in measuring the behavioural responses to a predator threat would improve our understanding of how *A. chrysopterus* navigate their ecological context in order to survive (Radford et al. 2014; Nedelec et al. 2016; Popper & Hawkins, 2018).

### 3.2.3 Scope for personality

As mentioned previously, personality can form an important source of behavioural variation in fish, including in clownfish (Sprenger et al. 2012; Schmiege et al. 2017). Fish that live in dynamic social groups are affected by the characteristics of particular individuals. For example, the proportions of bold or shy individuals can alter the social structure and communication between group members (Freeberg & Harvey, 2008). These personalities can consistently differ between individuals across differing contexts. Predation pressure is one such context, and in the case of alarm calling, personality can inform which individuals are likely to vocalise and which may remain silent (Van Oers et al. 2005; Seyfarth & Cheney, 2010). Perhaps only the

boldest of dominant females alarm call. This behavioural variation can also be influenced by previous experience and learning. Terrestrial animals demonstrate the capacity to distinguish not only between predator and non-predator, but between predator types, fine tuning their alarm calls with increasing experience of the stimulus (Seyfarth & Cheney, 2010). This capacity for associative learning depends therefore on previous experiences to formulate adaptive anti-predator responses in fish and influence an individual's behavioural responses (Chivers, et al., 2014). Greater focus, therefore, on the individual could prove informative and provide this additional insight.

### 3.2.4 Multimodal communication

Studying the function and ecology of individual sensory systems is well established in marine organisms (Hays et al. 2016). Once dominated by visual sensory ecology, audition and olfaction are fields of research that are experiencing increasing attention and appreciation. The oceans are no longer the "Silent World" as described by Jacques Cousteau in the 1950s. The challenge to resolve is that marine animals rely on complex multimodal sensing to inform behaviours, be it for navigation, evading predators, intercepting prey, mate selection or reproductive success (van der Sluijs et al. 2011; Higham & Hebets, 2013; Hays et al. 2016). The external environment has been found to alter the reliance on different sensory stimuli in fish, such as turbidity altering reliance on olfactory and visual cues in sticklebacks (van der Sluijs et al. 2011). Fish have been found to respond more strongly to multiple alarm cues presented together than if presented individually (McCormick & Manassa, 2008). While the information received and relative importance of chemical and visual cues in fish have been explored across many studies, acoustic communication has rarely been included (Helfman, 1989; Turner & Montgomery, 2003; McCormick & Manassa, 2008). This highlights the importance of incorporating acoustic signals into a multimodal approach to research for all fish systems, especially in the context of anthropogenically shifting environmental conditions (van der Sluijs, et al., 2011).

### 3.2.5 Specificity of alarm calls

Terrestrial examples demonstrate that there is strong selection pressure to communicate the level of threat, known as the 'threat-sensitive predator avoidance

hypothesis' that provides information on the degree of urgency of a threat (Brown et al. 2006; Freeberg & Harvey, 2008). Fish have been shown to emit differing aggressive sounds when directed at either conspecifics or heterospecifics, suggesting the capacity for specificity in their acoustic signalling (Myrberg, 1981). It would be of interest to test whether urgency is indicated in approaching predator models and the resulting behavioural responses as well as whether predator type might influence the alarm call given. Do clownfish alarm call differently to sharks, jacks and groupers as vervet monkeys call to snakes, eagles and leopards (Seyfarth & Cheney, 2010)?

## 3.3 Conclusion

This study looked at acoustic communication in the context of predation in the clownfish *A. chrysopterus*, finding that individuals use call production, call usage and call response, altering their acoustic and anti-predator behaviours when threatened by a predator. *A. chrysopterus* tended to respond when exposed to conspecific alarm calls. Responses varied between individuals and across different life stages. Such variation may, in part, potentially be explained by the scope of environmental conditions and individual experience and learning that favours flexibility in alarm calling for successful survival and reproduction (Seyfarth & Cheney, 2010). There are multiple detailed studies of the vocal behaviour in clownfish which have, until now, focussed solely on antagonistic and reproductive interactions; the majority within a laboratory setting (Parmentier et al. 2005, 2007, 2009; Colleye et al. 2009, 2011; Colleye & Parmentier, 2012). This study therefore not only explores an area of acoustic behaviour linked directly to survivorship (as with any adaptive anti-predator strategy) within a natural free-ranging context, but it also explores a new avenue in the acoustic ecology of clownfish, and coral reef fish in general. While fish have been known to vocalise since the days of Aristotle (350 BC), this study forms a contributing piece to the further understanding of acoustic communication.

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